UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DOS ALIMENTOS

Patrícia Acosta Caetano

DETERMINAÇÃO DE COMPOSTOS BIOATIVOS EM SISTEMAS MICROALGAIS

Santa Maria, RS
2022

Patrícia Acosta Caetano

DETERMINAÇÃO DE COMPOSTOS BIOATIVOS EM SISTEMAS MICROALGAIS

Dissertação apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciência e Tecnologia dos Alimentos.**

Orientadora: Profª. Dra. Leila Queiroz Zepka

Santa Maria, RS
2022

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

Acosta Caetano, Patrícia
Determinação de compostos bioativos em sistemas microalgais / Patrícia Acosta Caetano.- 2022.
110 p.; 30 cm

Orientador: Leila Queiroz Zepka
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Ciência e Tecnologia dos Alimentos, RS, 2022

1. biomoléculas 2. compostos secundários 3. bioprocessos microalgais I. Queiroz Zepka, Leila II. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, PATRíCIA ACOSTA CAETANO, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Dissertação) foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de qualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

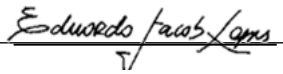
Patrícia Acosta Caetano

DETERMINAÇÃO DE COMPOSTOS BIOATIVOS EM SISTEMAS MICROALGAIS

Dissertação apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciência e Tecnologia dos Alimentos**.

Aprovado em 23 de março de 2022:

Leila Queiroz Zepka, Prof. Dra. (UFSM)
(Presidente/Orientador)



Eduardo Jacob Lopes, Prof. Dr. (UFSM)

Mariana Manzoni Maroneze, Dra. (EXTERNO)

Juliano Smanioto Barin, Prof. Dr. (UFSM)
(Membro suplente)

Santa Maria, RS
2022

A minha família.

AGRADECIMENTOS

À Deus, por iluminar-me sempre em qualquer trajetória, e me abençoar com tantas conquistas até hoje.

Àqueles que oportunizaram minha caminhada academia, Prof. Dra. Leila Queiroz Zepka e Prof. Dr. Eduardo Jacob Lopes, pela orientação, generosidade, paciência, disponibilidade, e pelos conhecimentos transmitidos.

Aos demais professores, por todo conhecimento adquirido ao longo dessa trajetória, seja em disciplinas, palestras ou até mesmo dúvidas e conversas pelo corredor. Grato pelo aprendizado.

Aos professores Roger Wagner e Cristiano Ragagnin, pelo empréstimo de equipamentos, tornando parte de minha pesquisa possível.

À minha família, pelo amor incondicional, confiança e apoio contínuo.

A minha irmã Larissa, por me apoiar nas dificuldades da vida, me confortar quando preciso, pelo carinho, pela amizade, te amo. E ao Zézinho, irmão casula, pelo amor e carinho, abraços de amor.

Aos meus pais Marivone e Benites, por todo amor incondicional, educação, apoio, por sempre acreditarem em minha capacidade e incentivar apoiar minhas decisões, amo vocês.

À minha amiga Nai pelo apoio, amizade, carinho, parceria, amor, pelas injeções diárias de ânimo, pela paciência, seus conselhos de vida e por proporcionar momentos incríveis juntas.

A todos os amigos do NTA por tornarem esta etapa final e os dias mais leve, pela amizade, pelo carinho e pela compreensão.

Às minhas amigas e colegas da sala de estudos e da pós-graduação, Paola e Patrícia Arrojo, pela amizade, companheirismo, pelos abraços, por nunca me deixarem perder a fé e por saber por tornarem meus “dias de laboratório” mais prazerosos.

A minha pesquisadora preferida Pricila Nass Pinheiro, por me proporcionar todo o conhecimento adquirido, sua paciência sem igual, por estar sempre comigo, inclusive feriados e finais de semana, sempre com novidades, e disposição para trabalhar, muitas vezes dando injeções de incentivo, aprendendo e ensinado, foi uma troca que serei grato eternamente, inclusive pelos posos, experimentos e refeições.

Aos ICs Richard e Luiza, por em pouco tempo proporcionar uma ligação, interação e energia incrível. Além de muito trabalho, os dias com vocês é gratificante.

Às amigas e colegas Andrêssa, Tatiele, Rafaela, Karem e Andriéli, pela troca de conhecimento, sendo também uma inspiração, pelo carinho, risadas, amizade e palavras de conforto.

As minhas amigos e primos fora da bolha, por sempre me apoiarem e estarem presente nos momentos bons e ruins dessa trajetória, pela amizade, pelo amor e alegria distribuídos sempre que é possível estarmos juntos.

Aos colegas do NTA, pelo companheirismo e ajuda sempre que necessário.

À CAPES, pela bolsa de mestrado concedida. E demais órgãos, fomentadores.

À Universidade Federal de Santa Maria, pela formação proporcionada.

Muito obrigada!

Tudo o que chega, chega sempre por alguma razão.
(Fernando Pessoa)

RESUMO

DETERMINAÇÃO DE COMPOSTOS BIOATIVOS EM SISTEMAS MICROALGAIS

AUTOR: Patrícia Acosta Caetano
ORIENTADORA: Leila Queiroz Zepka

Microalgas são fonte diversificada de moléculas bioativas, e são amplamente utilizados na indústria de alimentos, química e farmacêutica. Os carotenoides são importantes compostos conhecidos por suas atividades bioativas. Entre os carotenoides mais conhecidos o β-caroteno é o mais utilizado comercialmente, no entanto a luteína vem crescendo positivamente no mercado, principalmente por ser o único carotenoide a acumular-se em local específico no corpo humano, acumulando-se na mácula ocular. As microalgas metabolizam carotenoides comumente produzidos por cultivos fotoautotróficos, por outro lado, o metabolismo heterotrófico para a produção de pigmentos naturais tem atraído muita a atenção para aplicações comerciais, por superar dificuldades associadas ao fornecimento de CO_2 e luz, além de evitar os problemas de contaminação. Neste sentido, o objetivo do trabalho foi avaliar a (i) versatilidade metabólica da microalga *Chlorella vulgaris* em diferentes sistemas microalgais e (ii) avaliar a dinâmica dos carotenoides em função da curva de crescimento no cultivo heretotrófico, como fonte de carbono exógeno, a glicose. Os resultados demonstraram um perfil de 23 compostos com mudanças significativas nos tempos 0, 4, 12 e 96h qualitativas e quantitativas, alcançando uma biomassa celular máxima de 2200mg.L^{-1} e produtividade de 12.15mg.L.h^{-1} e observou-se que nas primeiras 4 horas um aumento significativo do conteúdo total de carotenoides em 1612,02μg.g^{-1}. Os carotenoides majoritários em todos extratos foram all-*trans*-luteína e all-*trans*-β-caroteno. Através dos resultados encontrados, os carotenoides demonstraram ser sintetizados no escuro e o cultivo heterotrófico ser uma opção interessante para a produção comercial de luteína, uma vez que possuem vantagens com alto conteúdo de luteína livre e uma maior taxa e crescimento.

Palavras-Chave: biomoléculas, compostos secundários, bioprocessos microalgais.

ABSTRACT

DETERMINATION OF BIOACTIVE COMPOUNDS IN MICROALGAE SYSTEMS

AUTHOR: Patrícia Acosta Caetano
ADVISOR: Leila Queiroz Zepka

Microalgae are a diverse source of bioactive molecules and are widely used in the food, chemical and pharmaceutical industries. Carotenoids are important compounds known for their bioactive activities. Among the best-known carotenoids, β-carotene is the most used commercially. However, lutein has been growing positively in the market, mainly because it is the only carotenoid able to accumulate in a specific location inside the human body, the eye macula. Microalgae are recognized as natural sources of carotenoids, which are commonly produced by photoautotrophic cultures. On the other hand, heterotrophic systems for the production of natural pigments have been showing potential for commercial applications, as they help to overcome difficulties associated with CO_2 supply and light incidence. In addition, heterotrophic models contribute to the prevention of contamination issues. In this sense, this work intended to evaluate the (i) metabolic versatility of the microalgae *Chlorella vulgaris* in different microalgal systems and (ii) to evaluate the behavior of carotenoids as a function of the growth curve in the heterotrophic cultivation, as a source of exogenous carbon, glucose. The results showed a profile of 23 compounds with significant qualitative and quantitative changes at times 0, 4, 12, and 96 h, reaching a maximum cell biomass of 2200mg.L^{-1} and productivity of 12.15 mg.L.h^{-1} and it was observed a significant increase in total carotenoid content by 1612.02μg.g^{-1} in the first 4 hours. The main carotenoids in all extracts were all-*trans*-lutein and all-*trans*-β-carotene. According to these results, it was verified that carotenoids can be synthesized in the dark and that heterotrophic cultures are interesting options for commercial production of lutein, since they show advantages, such as high content of free lutein and better growth rate.

Keywords: biomolecules, secondary compounds, microalgae bioprocesses.

LISTA DE FIGURAS

CAPITULO 1

CAPITULO 2

LISTA DE TABELAS

CAPITULO 2

SUMÁRIO

1 INTRODUÇÃO

Os compostos bioativos microalgais, destacando os carotenoides, promovem uma modulação no sistema processos biológico que resultam na promoção de uma melhor saúde por causa das propriedades terapêuticas. As principais formas de inserção comerciais são nas indústrias alimentícias, químicas e ciências farmacêuticas (Costa et al. 2020).

A biomassa microalgal são obtidas por condições de crescimento metodológico variado. Em geral, existem duas vias principais de fixação de carbono em microalgas. O cultivo fotoautotrófico corresponde ao crescimento fotossintético, é o procedimento mais comum empregado no cultivo de microalgas. Mas, uma alternativa viável para culturas fototróficas, mas restrita a poucas espécies de microalgas, é o uso de cultivos mixototróficos e heterotróficos (Canelli et al., 2022).

Com isso, obtemos carotenoides exclusivos, incluindo β-caroteno, α-caroteno, zeaxantina, luteína, violaxantina, neoxantina, mixoxantofila, equinenone e cantaxantina. Esses pigmentos possuem uma classificação genética relativamente simples e um curto ciclo celular. Assim, podemos afirmar que, o teor de carotenoides extraídos de microalgas é extremamente superior aos obtidos por fontes convencionais (Nascimento et al., 2020).

Seus metabolitos secundários vão além, com uma vasta produção de biomoléculas de diferentes classes químicas como álcoois, aldeídos, cetonas, hidrocarbonetos, ésteres, terpenos e compostos de enxofre (Santos et al., 2016b; Hosoglu, 2018), consideradas de relevância indústrial. Sistemas à base de microalgas descobrem centenas de novos compostos que são vendidos a preços 1000 vezes mais altos do que os de químicos sintéticos (Santos et al., 2016b).

A composição qualitativa e quantitativa desses bioativos em determinada matriz é dinâmica e depende de várias condições durante o cultivo/produção, armazenamento, pós-colheita e processamento. Uma biotecnologia microalgal bem-sucedida depende principalmente da escolha da cepa certa com ótimas condições de cultivo e técnicas analíticas. Portanto, desenvolvimento de diferentes vias metabólicas deve ser dotadas e exploradas para novos bioativos e de fontes naturais e sustentáveis (Geada et al., 2018; Vieira et al., 2019; Deprá et al., 2020).

Primeiramente, a aplicação de microalgas se sucedeu como fonte promissora para a produção sustentável de biocombustível, mas seu principal potencial é para produtos de alto

valor agregado como pigmentos, ácidos graxos, vitaminas, proteínas/enzimas e esteróis (Moradi, P., & Saidi, M. 2022).

O uso comercial de microalgas como fontes de produtos químicos específicos com *D. salina* para a produção de β-caroteno na década de 1970 (Borowitzka e Borowitzka, 1988), seguido pelo uso de *Haematococcus pluvialis* como fonte de astaxantina (Lorenz e Cysewski 2000) e *Crypthecodinium cohnii* para ácido graxo poliinsaturado de cadeia longa (PUFA) e ácido docosahexaenóico (DHA) (Kyle et al. 1998).

Recentemente, a biomassa microalgal de *Spirulina* e *Chlorella vulgaris* surgiu com perfil bioquímico atrativo, tornando-se muito utilizada em processos de base biológica. as principais espécies adicionadas em produtos de suplementos nutricionais como comprimidos, cápsulas ou em pó e promovidas como “superalimento”, “rico em compostos bioativos” (Caporgno e Mathys, 2018, Lafarga, 2019). Os compostos bioativos são moléculas que demonstram potencialidade na instrumentalização de diferentes tratamentos, com influência na ingestão energética, reduzindo o estado pró-inflamatório, estresse oxidativo e distúrbios metabólicos (Siriwardhana et al., 2013).

Este estudo fornece uma sistematização abrangente do conhecimento do estado atual da aplicação direta dessas técnicas na biotecnologia de microalgas, bem como tendências e desafios futuros em relação a dinâmica de produção e aplicação industrial. (Geada., 2018, Jabob-Lopes et al., 2019)

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o perfil de carotenoides na biomassa de *Chlorella vulgaris* em função da curva de crescimento, em cultivo heterotrófico utilizando glicose como fonte de carbono exógeno.

2.2 Objetivos específicos

1. Determinar a curva de crescimento da microalg*a Chlorella vulgaris* em cultivo heterotrófico;
2. Calcular parâmetros cinéticos de crescimento celular;
3. Obter os extratos de carotenoides da biomassa de todos os pontos da curva cinética da microalga;
4. Identificar e quantificar os extratos de carotenoides da biomassa em cultivo heterotrófico de todos os pontos da curva de crescimento do microrganismo por HPLC-PDA-MS/MS;
5. Verificar a relação entre cinética de crescimento e a produção de carotenoides.

CAPÍTULO 1
3 REVISÃO BIBLIOGRÁFICA

3.1. Microalgas

Microalgas são organismos fotossintéticos microscópicos com taxonomia complexa encontrados em diversos habitats aquáticos, geralmente são mais eficientes na conversão de energia solar em biomassa, principalmente devido à sua estrutura celular simples (CHACON-LEE; GONZALEZ-MARINO, 2010; LOURENÇO, 2006). Compreende um grupo diversificado de microrganismos com cerca de 72.500 espécies catalogadas de forma consistente, estes constituem um grupo polifilético e altamente diversificado de organismos procarióticos e eucarióticos. A classificação é baseada em várias propriedades, tais como pigmentação, natureza química do produto de armazenamento fotossintético, organização de membranas fotossintéticas e outras características morfológicas. (BOROWITZKA et al., 2018; RIZWAN, et al., 2018).

Os padrões taxonômicos atuais incluem 16 classes desses organismos. Entre essas classes, as mais abundantes são as diatomáceas (*Bacillariophyceae*), as algas verdes (*Chlorophyceae*) e as algas douradas (*Chrysophyceae*). Por outro lado, algas verdes, cianobactérias (*Cyanophyceae*) e diatomáceas são as mais significativas em termos de exploração e uso biotecnológico, conforme exposto na Tabela1. (BOROWITZKA et al., 2018). Microalgas verdes (clorofíceas), compartilham o mesmo mecanismo fotossintético que as plantas superiores, apresentam grande variedade nos níveis de organização, desde unicelulares, microalgas flageladas ou não, até talos morfologicamente complexos.

Essa divisão reúne a classe mais diversificada de microalgas e estão entre as linhagens que vêm sendo utilizada na produção de biomassa e compostos celulares, devido ao interesse global na exploração dos processos e produtos baseados em microalgas, fundamentalmente apoiados na composição química da biomassa de microalgas (proteínas, lipídios e pigmentos) além dos compostos extracelulares (carboidratos, lipídios e compostos orgânicos voláteis - COVs) excretados pelas culturas (JACOB-LOPES et al., 2006; QUEIROZ et al., 2007; JACOB-LOPES et al., 2007; JACOB-LOPES et al., 2008; ZEPKA et al., 2008; JACOB-LOPES et al., 2009; JACOB-LOPES et al., 2010; ZEPKA et al., 2010; QUEIROZ et al., 2011; RODRIGUES et al. 2015; SANTOS et al., 2016; MARONEZE et al, 2016; PATIAS et al., 2017).

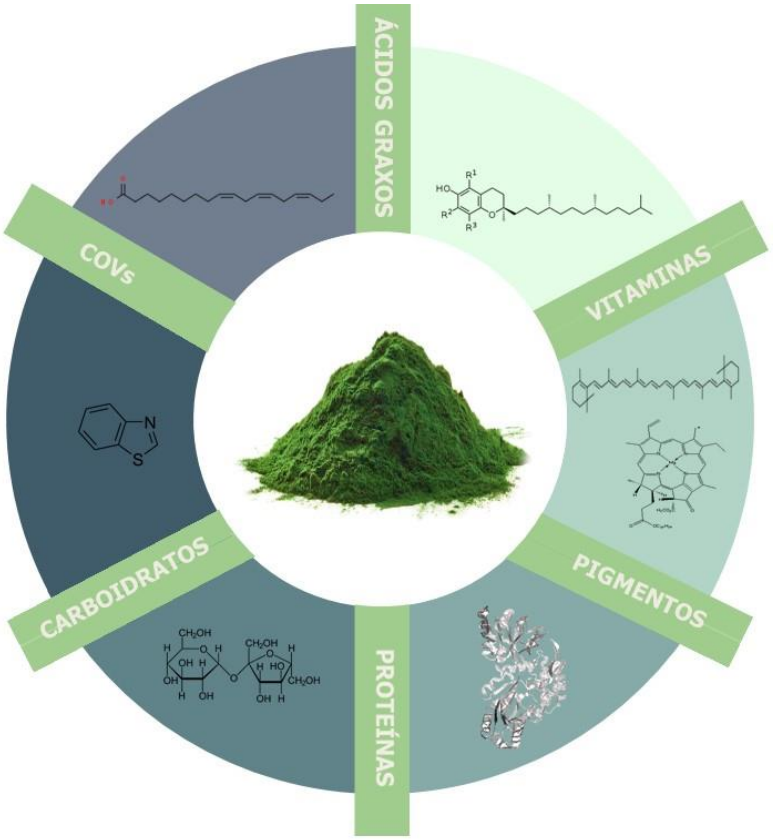


Figura 1. Principais compostos bioativos extraídos da biomassa microalgal.

Devido à sua versatilidade em se adaptar a uma ampla gama de condições de crescimento, climas e pH variado, as microalgas exibem uma clara vantagem sobre as plantas superiores para produção de pigmentos naturais. Os carotenoides com atividade bioativa comumente presentes e microalgas são astaxantina, β-caroteno, luteína, licopeno e cantaxantina. Apesar das vantagens percebidas, a fabricação em larga escala e econômica dos carotenoides por microalgas é atualmente bastante desafiadora tanto em termos de produção quanto de extração e purificação (GONG & BASSI, 2016).

Dentre as diversas microalgas na linhagem das clorofíceas, a *Chlorella vulgaris* é uma espécie de alga de água doce, amplamente cultivada, com produtividade elevada de biomassa (LIU & CHEN, 2014; KIM, 2016). Estudos relataram que a *Chlorella Vulgaris* é estabelecida como uma boa fonte de proteínas e pigmentos naturais como os carotenoides (SAFI et al., 2014). O conteúdo proteico pode chegar a 58% do peso seco de biomassa, e o perfil de aminoácidos essenciais das proteínas extraídas se compara bem aos padrões recomendados pela OMS/FAO (ZAGHDOUDI et al., 2015; KULKARNI & NIKOLOV, 2018). O conteúdo de luteína em *Chlorella sp*. pode atingir concentrações altas de até 7 mg/g de peso seco de

biomassa (SAFAFAR et al., 2016). Quase todas as espécies de *Chlorella* já relatadas são capazes de crescer de forma robusta sob condições heterotróficas com a adição de fontes de carbono orgânico, este tipo de cultivo é recomendado para a obtenção de produtos de alto valor, como os carotenoides.

3.1.1 *Chlorella*

Chlorella é um grupo de microalgas verdes eucarióticas com alta capacidade de fotossíntese. Através da fotossíntese eficiente, a *Chlorella* é capaz de se reproduzir dentro de algumas horas, exigindo apenas luz solar, dióxido de carbono, água e uma pequena quantidade de nutrientes. Quase todas as espécies de *Chlorella* relatadas são capazes de crescer de forma robusta sob condições heterotróficas com a adição de fontes de carbono orgânico, especialmente mono e dissacarídeos. As microalgas possuem tamanho pequeno, crescimento rápido, ciclo reprodutivo curto e forte adaptabilidade ao ambiente e, portanto, tem sido empregada como um organismo modelo para investigar os mecanismos de biossíntese para produção de metabólitos de alto valor (GERKEN et al., 2013; LIU & CHEN et al., 2014).

Comumente, as células de *Chlorella* são esféricas ou elipsoidais e o tamanho das células pode variar de 2 a 15 μm de diâmetro. São amplamente distribuídos em diversos habitats, como água doce, água marinha, solo. Se reproduz através da produção assexuada, quando crescido, os autospores são liberados simultaneamente através da ruptura da parede celular (GERKEN et al., 2013). *Chlorella* possui a parede celular espessa e rígida, mas a estrutura da parede celular pode diferir muito entre as espécies. Quando transferida para condições de estresse (por exemplo, escuro, privação de nitrogênio, adição de fonte de carbono exógeno), a parede celular engrossa e o cloroplasto começa a regredir ao estágio de proplastídio com uma redução gradual no número de tilacaróides, acompanhado pelo acúmulo de corpos lipídicos no citoplasma (YAMADA & SAKAGUCHI, 1982; LIU & CHEN et al., 2014)

O crescimento requer nutrientes, incluindo carbono, nitrogênio, fósforo, enxofre e metais. O carbono é o elemento predominante no crescimento da *Chlorella*, depende do cultivo, seja foto, mixo ou heterotrófico (SHI et al., 2000). O ar atmosférico contém apenas 0,04% de CO_2, não é suficiente para manter o rápido crescimento de *Chlorella* para alta densidade celular. Portanto, um suprimento deve ser normalmente fornecido às culturas, porém níveis muito elevados de carbono, podem causar uma diminuição no pH do meio e, assim, inibir ou até bloquear o crescimento de algas (ONG et al., 2010; CHIU et al., 2008).

A competitividade da produção heterotrófica de *Chlorella* sobre a produção fotoautotrófica está em grande parte com alta densidade celular e grande produtividade de

biomassa, e economia de luz. A alta densidade celular e a produtividade de biomassa da *Chlorella* heterotrófica podem ser alcançadas empregando-se estratégias de cultura com lotes alimentados, contínuos e reciclados (CHEN, 1996; CHEN & WALKER, 2012).

Carotenoides comumente encontrados em *Chlorella* incluem α e β-carotenos, luteína, zeaxantina, violaxantina e neoxantina. Os carotenoides têm importantes aplicações nas indústrias alimentícia, nutracêutica e farmacêutica devido à sua forte capacidade de coloração, e capacidade bioativa apresentando efeitos benéficos à saúde humana (FRASER & BRAMLEY, 2004). Usando *Chlorell*a como produtores de luteína e astaxantina foi proposto. Shi & Chen (1999), em estudos relatam espécies de *Chlorella* como uma ótima produtora de luteína em cultivo heterotrófico e mixotrófico, o foco nesses organismos e o emprego de estratégias de cultivo sugerem explorar o cultivo heterotrófico como fonte de carotenoides.

3.1.3 Cultivo heterotrófico

Diversas pesquisas têm sido realizadas com foco nos tipos de cultivos para aumentar a produtividade de biomassa com sustentabilidade. Os mais abordados, tem sido o fotoautotrófico, heterotrófico e o mixotrófico (MARONEZE et al. 2016; QUEIROZ et al., 2011; ZHAN, RONG & WANG, 2016; HU et al., 2018).

As vias metabólicas estão relacionadas à disponibilidade e aos tipos de nutrientes, nos meios em que microalgas estão sendo cultivadas. A glicose é a fonte orgânica preferida, com um rendimento de crescimento para *Chlorella* spp. heterotrófica de 0,35g biomassa·g açúcar $^{-1}$ (Ruiz et al., 2022).

Em geral, existem duas vias principais de fixação de carbono em microalgas. O cultivo fotoautotrófico corresponde ao crescimento fotossintético, é o procedimento mais comum empregado no cultivo de microalgas. Por serem microrganismos fotossintetizantes, as microalgas produzem energia através da luz e fixação de CO_2 atmosférico como fonte de carbono inorgânico através do ciclo de Calvin-Bensen (CHEN, 2006; WU et al., 2017). No entanto, o cultivo de microalgas usando o modo fotoautotrófico tem algumas limitações, maior custo com energia já que o lucro é o principal impulsionador do setor. No entanto, estamos longe de um consenso sobre o modo de produção mais econômico (Ruiz et al., 2022).

Enquanto alguns autores reivindicam a produção heterotrófica como o método mais viável em termos de custo. Uma alternativa viável para culturas fototróficas, mas restrita a poucas espécies de microalgas, é o uso de cultivos heterotróficos (CHEN, 2006). O metabolismo heterotrófico apresenta como características a ausência total de luminosidade e o

emprego de uma fonte de carbono orgânica exógena utilizada na obtenção de energia. Este tipo de cultivo permite o crescimento de microalgas em meios suplementados com fontes de carbono como: glicose, frutose, sacarose, acetato e glicerol, além da utilização direta de águas residuais (FRANCISCO et al., 2014; SANTOS et al., 2016; PEREZ-GARCIA et al., 2011).

A glicose é a fonte de carbono exógena mais utilizada para as culturas heterotróficas microalgais, devido às elevadas taxas de crescimento e respiração obtidas com esse substrato. Em geral, quando se utilizam outros substratos, as microalgas requerem um período de adaptação, representadas pela extensa fase *lag*, necessária para a síntese das enzimas e dos sistemas de transporte específicos para a assimilação e o consumo das moléculas (PEREZ-GARCIA et al., 2011).

O glicogênio é o principal carboidrato de reserva, podendo, assim como a glicose exógena, ser convertido em glicose-6-fosfato e ser metabolizada pela via respiratória. Algumas enzimas do Ciclo de Krebs são detectadas com atividades extremamente baixas e o metabolismo no escuro está ligado à presença de oxigênio, sendo que a principal rota é a via da pentose-fosfato (FAY, 1983). A glicose-6-fosfato é oxidada e descarboxilada em ribulose-5-fosfato. As reações são catalisadas pela glicose 6-fosfato-desidrogenase e 6-fosfo-gluconato-desidrogenase, respectivamente. Ambas as enzimas estão presentes em altas concentrações nas cianobactérias e duas moléculas de NADPH (nicotinamida adenina dinucleótido fosfato hidreto) são geradas, com subsequente oxidação na cadeia respiratória, rendendo 2 ATPs (adenosina trifosfato) (FAY, 1983; PEREZ-GARCIA et al., 2011).

Análises dos extratos celulares revelaram que a ribulose-1,5-difosfato inibe a glicose-6-fosfato-desidrogenase, a primeira enzima da via oxidativa (HU et al., 2018). A transferência das culturas para cultivo no escuro está ligada ao imediato desaparecimento deste metabólito, com ativação da via metabólica oxidativa (PELROY; BASSHAM, 1972).

Este tipo de cultivo tem a vantagem de acelerar a taxa de crescimento celular, aumentar o acúmulo de biomassa em comparação com a cultura autotrófica e até mesmo pode utilizar substratos de baixo custo como resíduos agroindustriais, reduzindo o custo global do processo (FRANCISCO et al., 2014). O custo associado à aquisição da fonte de carbono e operação de equipamentos de cultivo heterotrófico pode ser compensado pela quantidade e qualidade dos bioprodutos gerados para aplicação industrial (HU et al., 2018).

Portanto, o cultivo heterotrófico serve como uma alternativa, para a obtenção de compostos naturais de interesse, que são principalmente de origem intracelular, e dependentes da via metabólica. (CÓRDOVA et al., 2018).

3.2 Compostos microalgais

Existe um interesse global na exploração dos processos e produtos baseados em microalgas, a biodiversidade, o estabelecimento de tecnologia de cultivo em grande escala, e a consequente variabilidade na composição química da biomassa de microalgas além dos compostos extracelulares excretados pelas células, vêm permitindo sejam utilizadas em diversas aplicações (BOROWITZKA, 2013; BOROWITZKA, 2018). A produtividade das microalgas pode superar o de qualquer outra matéria-prima terrestre utilizada em processos de biorrefinaria, pois, além de possuir um cultivo mais rápido, tanto no processamento quanto na obtenção da biomassa, também podem ser cultivadas em águas residuais (RODRIGUES et al., 2014; GERARDO et al., 2015).

Microalgas eucarióticas possuem um elevado crescimento celular, e em paralelo, a biomassa produzida é uma excelente fonte diversificada de moléculas como lipídios, proteínas, polissacarídeos, antioxidantes, esteróis insaponificáveis, agentes antimicrobianos e minerais. Adicionalmente, esses microrganismos são uma fonte potencial de triacilglicerídeos que podem conter quantidades elevadas de ácidos graxos de cadeia longa poliinsaturados, tais como o ômega 3, ácido eicosapentaenoico (EPA) (HERRERO et al.,2015; RIZWAN et al., 2018).

Estes microrganismos contêm altos níveis de óleos, carboidratos e proteínas que os tornam matérias-primas versáteis para a produção de combustíveis e biogás em paralelo com produtos químicos valiosos para a alimentação humana e animal (SILVA et al., 2015; BRASIL et al., 2017; CÓRDOVA et al., 2018). A combinação da produção de biocombustíveis de microalgas com as aplicações convencionais é excelente para prosperar a indústria de biorrefinaria microalgal de forma sustentável (QUEIROZ et al., 2013; MA, et al., 2015). Observa-se também que, além das frações de metabolitos não volteis, o rendimento da microalga não satisfaz completamente o balanço de carbono total do sistema, sugerindo que parte deste balanço está direcionado para a produção de compostos orgânicos voláteis (JACOB-LOPES et al, 2010).

Microalgas e seus extratos representam uma fonte vasta e inexplorada de compostos com atividade biológica. As conversões de biomassa de microalgas, para produtos e compostos

de alto valor agregado estão globalmente ganhando um destaque significativo (HERRERO & IBÁÑEZ, 2018). Embora centenas desses metabólitos tenham sido identificados em culturas de microrganismos, a indução de síntese é na maioria dos casos, desconhecida, a separação e recuperação dos compostos precisam ser otimizadas e, assim, a inserção em produtos comerciais depende ainda de pesquisa e desenvolvimento (RAMÍREZ-MÉRIDA et al., 2017).

Em relação aos pigmentos que possuem propriedades bioativas, inclui-se três classes: carotenoides e clorofilas e ficobiliproteínas, pigmentos naturalmente presentes na biomassa microalgal que recebem cada vez mais atenção (RODRIGUES et al., 2015; D'ALESSANDRO & FILHO, 2016). Estes, já são aplicados na indústria de corantes, e pelo fato de apresentar diversas atividades biológicas, como: atividade antioxidante, efeitos anti-inflamatórios, redução da degeneração macular, atividades neuroprotetoras, de alguns desses compostos, novos estudos e aplicações estão surgindo, principalmente nos setores cosmético e farmacêutico (ZHANG et al., 2017; ZHANG et al., 2014).

Os carotenoides têm altos valores comerciais, especialmente por sua alta demanda como compostos bioativos. Assim, são atualmente os produtos mais comercializados a partir de microalgas, renovando o interesse em aumentar a pesquisa e o desenvolvimento desses compostos na biomassa de microalgas (Poojary et al., 2016). O mercado global de carotenóides foi de US$ 1,24 bilhões em 2016 e projeta-se aumentar para US $ 1,53 bilhões até 2021, com uma taxa de crescimento anual composta (CAGR) de 3,78% de 2016 a 2021(HU et al., 2018; JACOB-LOPES et al., 2019). As maiores participações de mercado são β-caroteno e astaxantina, com preço médio próximo a US $ 2.500 / kg (Suganya, Varman, Masjuki, & Renganathan, 2016).

3.2.1 Carotenoides em microalgas

Os carotenoides representam um grupo de pigmentos, sintetizados naturalmente por organismos fotossintéticos (GONG & BASSI, 2016). Quimicamente, são compostos isoprenóides lipofílicos, suas características colorem na faixa do amarelo ao vermelho, classificados de acordo com o número de carbonos que constituem sua estrutura. A maioria dos carotenoides compartilha a estrutura C40 comum das unidades de isopreno, sendo encontrada na natureza mais abundantemente. Além disso, suas estruturas químicas são constituídas por diversos grupos terminais (BRITTON et al., 2008; RODRIGUEZ-AMAYA, 2015).

Considerando a estrutura dos carotenoides, eles podem ser classificados em carotenos e xantofilas. Os carotenos são compostos que contêm apenas hidrocarbonetos em sua estrutura (por exemplo, β-caroteno e licopeno). Por outro lado, as xantofilas são carotenoides oxigenados, que contêm diferentes grupos funcionais, como grupos funcionais epoxídos, hidroxila, ceto e metoxi. Por sua vez, as xantofilas estão entre os principais carotenoides nos tecidos fotossintéticos. (GONG & BASSI et al., 2016).

De acordo com essas modificações, esses grupos funcionais contendo oxigênio afetam as funções biológicas e a solubilidade dos carotenoides, tornando as xantofilas mais polares que os carotenos, permitindo sua separação por meio de vários tipos de cromatógrafos (BRITTON et al, 2008). O conjunto de duplas ligações conjugadas *(c.d.b)*, é responsável pela cor e características como capacidade antioxidante e suas propriedades. Portanto a capacidade bioativa dos carotenoides das microalgas está diretamente ligada ao aumento da intensidade de absorção, chamado efeito batocrômico (RODRIGUEZ-AMAYA, 2016). Algumas estruturas podem ser visualizadas na Figura1.

Em microalgas os carotenoides têm funções primárias e secundárias. A primária atua sobre o sistema fotossintético celular, a secundária protege a clorofila do fotodano. Por essa razão, sob condições de estresse, os carotenoides secundários são sintetizados em grandes quantidades. Além disso, os carotenoides apresentam propriedades antioxidantes que protegem as microalgas de ataques dos radicais livres. (GUEDES et al., 2011; GONG & BASSI, 2016).

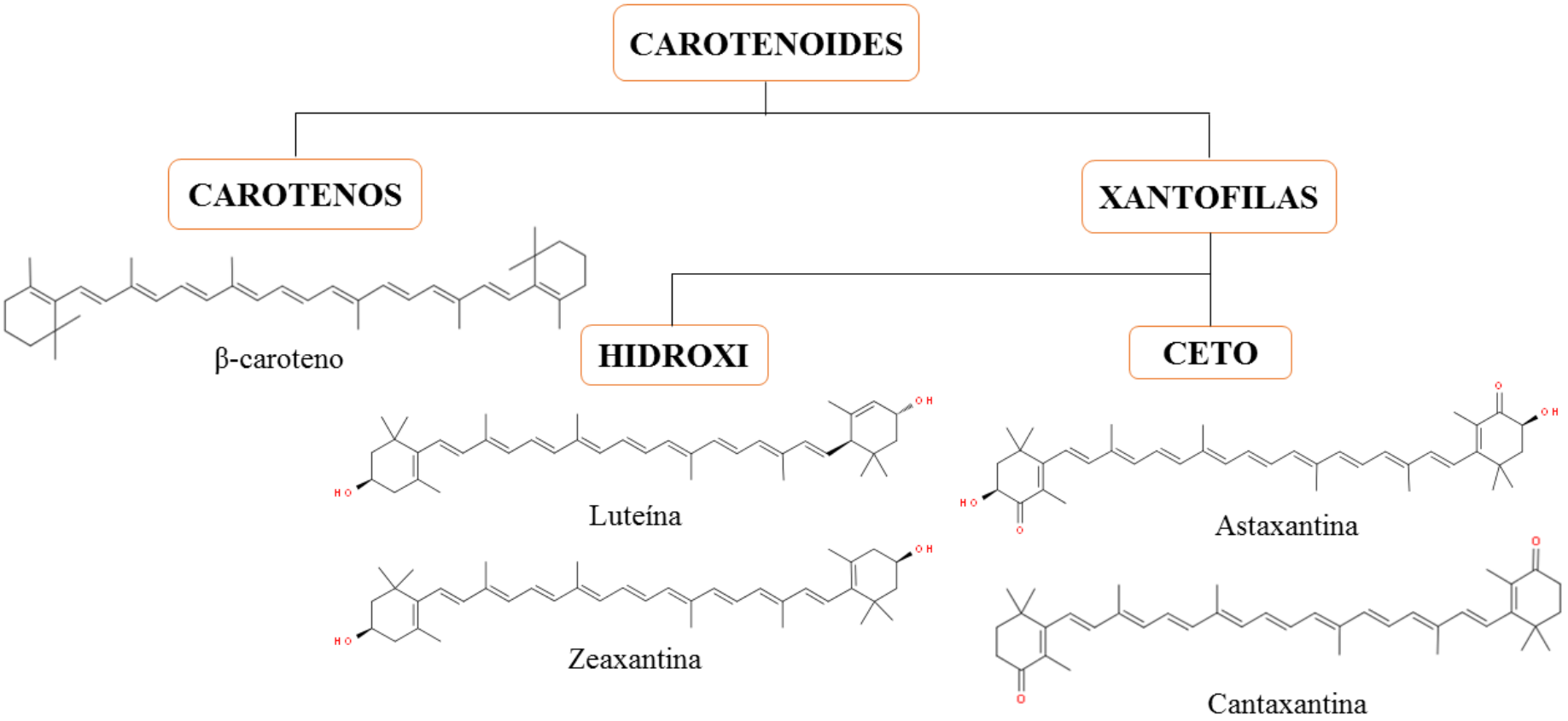


Figura 2. Carotenoides e xantofilas encontrados em microalgas.

Segundo Rodrigues et al. 2015, várias espécies de algas demonstram a capacidade de sintetizar carotenoides específicos. Entre os principais carotenoides produzidos por esses microrganismos se destacam o β-caroteno, luteína, violaxantina, zeaxantina, astaxantina, equinenona, cantaxantina e a neoxantina. Espécies específicas de algas verdes possuem xantofilas adicionais, como a loroxantina (BRITTON et al, 2004; TAKAISHI et al 2011; GOIRIS et al, 2012; RODRIGUES et al, 2015; CHEN et al., 2016).

A produção industrial de carotenoides naturais usando microalgas envolve, principalmente, duas espécies, *Dunaliella salina* e *Haematococcus pluvialis*, que produzem β-caroteno e astaxantina, respectivamente (PŘIBYL et al., 2016). Porém, existem deficiências inerentes a essas cepas, como crescimento lento, rendimento insuficiente, assim sendo estudos vem sendo realizados na busca por cultivos de microalgas alternativas que possam suplementar a produção de carotenoides. A microalga *Chlorella Vulgaris*, cresce rapidamente, possuem conteúdo substancial de carotenoides e realizam-se de forma robusta em biorreatores (KIM, 2016).

A produção destes compostos à base de microalgas em escala industrial surgiu como uma oportunidade comercial visando ganhar participação de mercado no segmento de moléculas com atividades biológicas, inicialmente dominado por moléculas sintéticas e de origem animal e vegetal (BOROWITZKA et al., 2018).Em face disso, o uso de corantes naturais tem aumentado constantemente principalmente devido a mudanças na preferência do consumidor em relação a produtos mais naturais conhecidos por exibir propriedades funcionais específicas (SINGH et al, 2015).

Os principais carotenoides de interesse comercial das microalgas são o β-caroteno, luteína, cantaxantina, fucoxantina e a astaxantina, apresentados na Tabela 2. A astaxantina e o β-caroteno são os dois carotenoides mais reconhecidos no mercado global, a astaxantina apresenta o maior potencial antioxidante em função do tamanho do cromóforo. β-caroteno, é responsável pela ação pró-vitamínica A (DUFOSSÉ et al., 2005). A luteína tem papel importante no mercado nutracêutico, uma vez que a ação sobre a saúde macular já foi comprovada, auxiliando no tratamento e prevenção de catarata e degeneração macular (CHEN et al., 2016).

O mercado global de luteína foi estimado em US$ 135 milhões em 2015 e continuará a crescer até 2024, com uma taxa de crescimento de 5,3% (Global Market Insights Lutein Market Report, 2016). O atual suprimento comercial de luteína é dependente apenas de pétalas de flores de calêndula, no entanto o rendimento é depende da variação sazonal (HU et al., 2018). Kamoshita et al., (2016) comprovou que a luteína retarda a progressão da degeneração macular relacionada à idade (DMRI), uma das principais causas de cegueira em pessoas mais velhas.

Essa xantofila que se acumulam na região macular da retina estão principalmente na forma all-*trans*-luteína, a biodisponibilidade da atual fonte produtora é em grande maioria ésteres de luteína. Portanto, a produção comercial de luteína está preocupada com o teor de luteína all-*trans*, e não com o teor total de luteína, mostrando que cepas de microalgas são ótimas fontes exploratórias para obtenção deste composto (LIN et al., 2015)

Finalmente, os compostos bioativos sintetizados por microalgas são de grande importância para o mercado industrial. Nesse sentido, a extensão em que a biossíntese dessas estruturas de microalgas é elucidada será útil nas indústrias. As microalgas apresentaram um perfil rico e interessante de carotenoides, caracterizado pela prevalência de xantofilas sobre carotenos, essas microalgas podem ser vistas como fontes sustentáveis de carotenoides na perspectiva de uma abordagem de biorrefinaria, onde vários componentes de biomassa são valorizados (DI LENA et al., 2018).

CAPITULO 2

4 – ARTIGO: INFLUENCE OF HETEROTROPHIC CULTURE FROM CAROTENOIDS PRODUCTION

O artigo será submetido a Food research international

Influence of heterotrophic culture on carotenoid production

Patricia Acosta Caetano[a], Álisson Santos de Oliveira[a], Tatiele Casagrande Nascimento[a], Pricila Nass Pinheiro[a], Andressas da Silva Fernandes[a], Eduardo Jacob-Lopes, Leila Queiroz Zepka[a]

[a] Department of Food Technology and Science, Federal University of Santa Maria (UFSM), P.O. Box 5021, Santa Maria 97105-900, Brazil

*Corresponding author: e-mail: zepkaleila@yahoo.com.br; Phone/Fax: +55-55-32208254

ABSTRACT: Carotenoid profile in Chlorella vulgaris biomass growth were evaluated from heterotrophic culture using glucose as exogenous carbon source. However, considering the growing market for lutein and the fact that microalgae produce higher amounts of free lutein compared to current sources. Carotenoids were determined by HPLC-PDA-MS/MS, and a total of 23 carotenoids were separatedThe results showed that at different cultivation times, the experiment at 4 hours of cultivation time differed significantly from times 0, 12 and 96 hours. The extract showed a total of 17 different carotenoids identified (Table 3), all-trans-lutein (707.30 $\mu g.g^{-1}$) was the major carotenoid, followed by all-trans-β-carotene (186.40 $\mu g.g^{-1}$). Additionally, it is worth noting that the total absence of light promoted the formation of all-trans-echinenone and 9-cis-echinenone, a microalgae carotenoid that is hugely revered due to its antioxidant potential provided by the conjugated double bonds present in its structure. Through the results found, carotenoids have been shown to be synthesized in the dark and heterotrophic cultivation seems to be an interesting option for the commercial production mainly of lutein, since they have advantages with high content of free lutein and a higher rate and growth.

Keywords: carotenoids, microalgae, heterotrophic culture, bioactive compounds.

1. Introduction

There is a global interest in the exploration and development of cultivation processes and extractions of bioproducts supported in microalgae. Fundamentally based on the production of intracellular compounds synthesized by these microorganisms, such as carotenoids (Rizwan et al., 2018). Microalgae of the genus *Chlorella vulgaris* are ideal for exploitation because they are extremely resistant to adverse and invasive conditions. In addition, they are marketed as a rich food additive and are considered safe food sources by GRAS status generally recognized as safe, according to the Food and Drug Administration (Chacón-Lee and González-Mariño, 2010; Mócsai et al., 2019).

Due to the significantly positive impact of carotenoids on commercial application, a growing demand for these biologically active compounds has increased the attention and commercial interest both in the supply and in obtaining promising natural carotenoids produced by the synthesis of microorganisms. The world market for carotenoids was estimated at US$ 1,577 million in 2017 and is expected to reach US$ 2,098 million by 2025. By 2017, astaxanthin, β-carotene, and lutein were the major contributors to the global carotenoid market in value terms. β-carotene and astaxanthin, with an average price close to US$ 2,500/kg (Patias et al., 2017; Jacob-Lopes et al., 2019).

Regardless of the intended application of biomass, the kinetic model of microalgae culture is significant to evaluate the proficiency and performance of a reactor, as well as the quantity and quality of the desired product. The growth curve of microalgae cultures shows different phases involving changes in the physical-chemical characteristics of the microalgae (Queiroz et al., 2011; Tijani et al., 2018; Tkáčová et al., 2018).

The heterotrophic culture maintained by sources of exogenous carbon is a potential form of pigment production. The choice of inputs that have a low cost to the culture medium is of great importance for process economics (Wen and Chen, 2003). The efficiency of converting incoming energy into the form of electricity in ATP and NADPH at heterotrophic cultivation is economically more advantageous than photoautotrophic cultivation. Glucose is the preferred source of carbon in *Chlorella* species in heterotrophic cultivation for the production of lutein (Perez-Garcia et al., 2011; Behrens et al., 2005).

The objective of this study was to determine the heterotrophic metabolic potential of the *Chlorella Vulgaris* microalgae for production of carotenoids, in different phases of microalgae growth kinetics in batch cultures.

2. Material and methods

2.1. Microorganisms and culture media

The axenic cultures of *Chlorella vulgaris* (CPCC90) was manipulated in the experiments. The culture in was disseminated and maintained in synthetic BG11 medium (Rippka, Deruelles, Waterbury, Herdman & Stanier, 1979). The incubation conditions were 25 ^{o}C, photon flux density of 15 μmol/m^2/s^{-1} and a photoperiod of 12/12 h light/dark were used.

2.2. Microalgal biomass production

The experiments were developed in a bioreactor operating in the batch system, fed with 2.0L of culture medium. The experimental conditions were as follows: initial inoculum concentration of 100mg/L, a temperature of 25°C, pH adjusted to 7.6, aeration of 1 volume of air per culture volume per minute and absence of light. The culture medium consisted of BG11 supplemented with an exogenous carbon source to obtain a fixed C/N ratio of 20. The monosaccharide concentration was adjusted stoichiometrically. The source and the concentration of organic carbon used were: D-glucose (12.4 g/L). The glucose was weighed and diluted in synthetic BG11 medium, followed by autoclaving at 121 ° C for 20 min (Francisco et al., 2014).

Cell biomass concentration, pH control and carotenoid production were monitored every 24h during growth kinetics of the microorganism. The experiments were performed in duplicate, so the kinetic data refer to the mean value of the replicates. The pH values were determined by potentiometer (Mettler-Toledo, São Paulo, SP, Brazil). Cell biomass was determined gravimetrically by filtering a known culture volume through a 0.45μm membrane filter (Millex FG®, Billerica, MA, USA), and drying at 60°C for 24h. The biomasses for pigment extraction were separated from the culture medium by centrifugation. It was subsequently lyophilized for 24h at -50°C above -175μm Hg and then stored under refrigeration until analysis.

2.3. Carotenoid extraction

The carotenoids were exhaustively extracted from the freeze-dried sample (0.1 ± 0.02g) with ethyl acetate and methanol in a mortar with a pestle followed by centrifugation (Hitachi, Tokyo, Japan) for 7min at 3500rpm (Patias et al., 2017). The extraction procedure was repeated

until the supernatant becomes colorless. The homogenized sample suspension was filtered through a 0.22μm polyethylene membrane, concentrated in a rotary evaporator (T<30°C), suspended in a mixture of petroleum ether/diethyl ether [1:1 (v/v)], and saponified overnight (16h) with 10% (w/v) methanolic KOH at room temperature. The alkali was removed by washing with distilled water, and each extract was once again concentrated in a rotary evaporator, flushed with N_2 and kept at −37°C in the dark until chromatographic analysis. All extractions were performed in triplicate.

2.4. HPLC-PDA-MS/MS analysis

The carotenoids were analyzed by high performance liquid chromatography, HPLC (Shimadzu, Kyoto, Japan) equipped with quaternary pumps (model LC-20AD) and an automatic injector. The equipment was connected in series to a PDA detector model SPD-M20A and a mass spectrometer with a triple quadrupole 8040 analyzer (Shimadzu, Kyoto, Japan) and APCI ionization source (Shimadzu, Kyoto, Japan). UV-visible spectra were obtained between 250 and 600nm, and the chromatograms were processed at 450nm. The mass spectra were obtained with a scanning range of m/z of 300 to 700nm. The HPLC-PDA MS/MS parameters were adapted as described previously by Bukowski et al (2018). The instrument was run in multiple reaction monitoring modes (MRM), with pre-cursor m/z, product m/z and collision energy voltages optimized. The parameters MS and MS/MS were as follows: positive mode; potential interface maintained at 4.0 KV; Temperature DL, 200°C; dry gas, N_2, with flow rate of 10 $\cdot min^{-1}$; nebulizer gas, argon with flow rate of 3 $L \cdot min^{-1}$; interface temperature, 300°C; Carotenoid separation was performed on an Acclaim ™ C30 reverse phase column, 5μm (250 × 4.6 mm) column (Thermo Fisher Scientific, Waltham, MA, USA).

Prior to HPLC–PDA–MS/MS analysis, the carotenoid extract was solubilized in methanol (MeOH): methyl-terbutylether (MTBE) (70:30) and filtered through Millipore membranes (0.22μm). The mobile phase consisted in a mixture of MeOH and MTBE. A linear gradient was applied from 95:5 to 70:30 in 30min, to 50:50 in 20min. The flow rate was 0.9mL/min. The identification was performed according to the following combined information: elution order on C30 HPLC column, co-chromatography with authentic standards, UV-visible spectrum (λ max, spectral fine structure, peak *cis* intensity), and mass spectra characteristics (protonated molecule ([M+H]+) and MS/MS fragments), compared with data available in the literature (Rodrigues et al., 2015; Van Breemen, Dong & Pajkovic, 2012; Zepka

& Mercadante, 2009; De Rosso & Mercadante, 2007; Britton, 1995; Patias et al., 2017; Huang et al., 2017). Carotenoids were quantified by HPLC-PDA using external calibration curves for all-trans-lutein and all-trans-β-carotene at five concentration levels. Total carotenoid content was calculated as the sum of the content of each individual carotenoid separated in column C30.

2.5. Statistical analysis

Descriptive statistics, one-way ANOVA and Tukey's test ($p < 0.05$) were applied to the experimental data. The analyzes were performed using Statistica 7.0 software (StatSoft, Tulsa-OK, US).

3. Results and discussion

1. Biomass Production

The heterotrophic growth of *C.vulgaris* in the medium containing 24.7g.L^{-1} glucose, applying a C/N ratio of 20 was achieved and the results are presented in Table1. In the absence of light, the use of suitable carbon source is crucial for attaining high biomass yield in algal culture. The maximum specific growth rate was 0.0248h^{-1}, similar to that obtained with the use of the same monosaccharide by Francisco et al. (2014) which was 0.0320h^{-1} in the study of different carbohydrates as a carbon source using cyanobacteria, resulting in a generation time of 27.94h^{-1}.

Table 1. Kinetics parameters of microalgae *Chlorella Vulgaris.*

Parameters	Results
μmax (h^{-1})	0.0248
tg (h^{-1})	27.94
Xmáx (mg.L^{-1})	2200
Px (mg.L^{-1})	12.15

μmáx maximum specific growth rate, tg generation time, Xmáx maximum cell biomass, Px biomass productivity.

In terms of maximum cellular biomass, the microalgae *Chlorella Vulgaris* presented satisfactory performance, when compared to the results obtained in the mixotrophic culture by Molazadeh et al., (2019), this being an average yield of 2200mg.L^{-1}. The higher biomass production is because of the elevated assimilation capacity of glucose with lower energy

expenditure via phosphorolytic degradation of the substrate. Biomass yield coefficients generally have lower values than one, but values greater than one, obtained in the experiment are due to the resulting energy savings of phosphorolysis of the substrate. In addition, considering the biomass productivity parameter, productivity was obtained around 12. 15mg.L.h^{-1}.

An active hexose/H^+ inducer system in *Chlorella* cells is found and some other genes, these are photosynthetically absent, and are activated when autotrophically cultured *Chlorella* cells change for growth heterotrophic in the presence of D-glucose, facilitating the absorption of available glucose as a substrate source (Hilgarth et al., 1991; Perez-Garcia, 2011). This sugar transporter mechanism is inactivated when the pH is below 6.0 (Komor and Tanner, 1976; Komor et al., 1979). Considering that the cell growth kinetics occurred up to 120h with a pH decrease during the experiment, and that in the decline phase the microalga was at pH 5.89, this may be one of the justifications for the cell death of the microorganism.

2. Identification of carotenoids

Naturally, the pigments are produced under photoautotrophic growth conditions, but some cultures are produced and in large amounts under heterotrophic dark conditions. A total of 23 carotenoids were separated in all experiments from *Chlorella vulgaris* (Fig. 1). The separated carotenoids were identified or tentatively identified (Table 1). A description of microalgae carotenoid identification using chromatographic information HPLC-PDA-MS/MS (APCI positive mode) was previously described in detail in the literature by Fernandes et al. (2021), Nascimento et al. (2021), Maroneze et al. (2020), Patias et al. (2017), Rodrigues et al. (2015, 2014).

Table 2. Chromatographic, UV–vis spectrum and mass characteristics, obtained by HPLC–PDA–MS/MS of *Chlorella vulgaris* carotenoids.

Peak[a]	Pigments	t_R (min)[b]	UV-vis characteristics			Fragment ions (positive mode) (*m/z*)	
			$\lambda_{máx}$ (nm)[c]	III/II[d] (%)	A_B/II[e] (%)	$[M + H]^+$	MS/MS
1	15-*cis*- neochrome	6.2	327, 405, 428, 455	65	42	601	583 $[M + H - 18]^+$, 565 $[M + H - 18 - 18]^+$, 547 $[M + H - 18 - 18 - 18]^+$, 509 $[M + H - 92]^+$
2	13-*cis*- neoxanthin	6.7	337, 419, 443,471	81	17	601	583 $[M + H - 18]^{+\cdot}$, 547 $[M + H - 18 - 18 - 18]^+$, 221
3	All-*trans*-neoxanthin	7.5	415, 438, 468	78	0	601	583 $[M + H - 18]^+$, 565 $[M + H - 18 - 18]^+$, 547 $[M + H - 18 - 18 - 18]^+$, 509 $[M + H - 92]^+$
4	all-*trans*-neochrome	7.7	398,421, 447	80	0	601	583 $[M + H - 18]^{+\cdot}$, 547 $[M + H - 18 - 18 - 18]^+$, 221
5	9-*cis*- neoxanthin	8.0	325, 415, 438, 467	78	0	601	583 $[M + H - 18]^+$, 565 $[M + H - 18 - 18]^+$, 509 $[M + H - 92]^+$
6	13-*cis*-antheraxanthin	8.7	326, 415, 438, 467	72	13	585	583 $[M + H - 18]^+$, 565 $[M + H - 18 - 18]^+$, 509 $[M + H - 92]^+$
7	all-*trans*-luteoxanthin	8.7	400, 420, 447	100	0	601	583 $[M + H - 18]^+$
8	all-*trans*-antheraxanthin	9.3	416, 442, 473	50	0	585	567 $[M + H - 18]^+$, 549 $[M + H - 18 - 18]^+$, 531
9	13-*cis*-lutein	10.3	331, 415, 437, 465	37	31	569	551 $[M + H - 18]^+$, 533 $[M + H - 18 - 18]^+$
10	15-*cis*-lutein	11.2	328, 415, 438, 465	25	39	569	551 $[M + H - 18]^+$, 533 $[M + H - 18 - 18]^+$, 477 $[M + H - 92]^+$
11	all-*trans*-lutein	12.3	419, 443, 471	57	0	569	551 $[M + H - 18]^+$, 533 $[M + H - 18 - 18]^+$
12	13-*cis*-zeoxanthin	13.2	326, 420, 440, 465	25	nc[f]	569	551 $[M + H - 18]^+$, 533 $[M + H - 18 - 18]^+$, 495, 477 $[M + H - 92]^+$, 459
13	all-*trans*-zeaxanthin	14.5	425, 449, 475	25	0	569	551 $[M + H - 18]^+$, 533 $[M + H - 18 - 18]^+$,

							495, 477 $[M + H - 92]^+$, 459
14	9-*cis*-lutein	15.2	326, 420, 440, 465	50	12	569	551 $[M + H - 18]^+$, 533 $[M + H - 18 - 18]^+$, 495, 477 $[M + H - 92]^+$, 459
15	9-*cis*-zeaxanthin	17.6	338, 420, 445, 470	33	25	569	551 $[M + H - 18]^+$, 533 $[M + H - 18 - 18]^+$, 495, 477 $[M + H - 92]^+$, 459
16	2'-dehydrodeoxymyxol	20.2	445, 473, 504	63	0	567	549 $[M + H - 18]^+$
17	5,6-epoxy-β-carotene	20.2	420, 446, 470	50	0	553	535 $[M + H - 18]^+$, 461 $[M + H - 92]^+$, 205
18	all-*trans*-echinenone	23.6	462	nc	0	551	533 $[M + H - 18]^+$, 427, 203
19	9-*cis*-echinenone	25.6	342, 450	nc	20	551	533 $[M + H - 18]^+$, 427, 203
20	all-*trans*-α-carotene	27.8	420, 445, 473	62	0	537	444 $[M + H - 92]^+$, 399, 355
21	9-*cis*- α-carotene	28.9		70	nc		
22	all-*trans*-β-carotene	31.0	425, 451, 476	25	0	537	444 $[M + H - 92]^+$, 399, 355
23	9-*cis*-β-carotene	33.7	341, 420, 446, 472	20	14	537	444 $[M + H - 92]^+$, 399, 355

[a]Numbered according to the chromatograms shown in Figure 1. [b]t_R: Retention time on the C_{30} column. [c]Linear gradient Methanol: MTBE. [d]Spectral fine structure: Ratio of the height of the longest wavelength absorption peak (III) and that of the middle absorption peak (II). [e]Ratio of the *cis* peak (A_B) and the middle absorption peak (II). [f] Not detecte

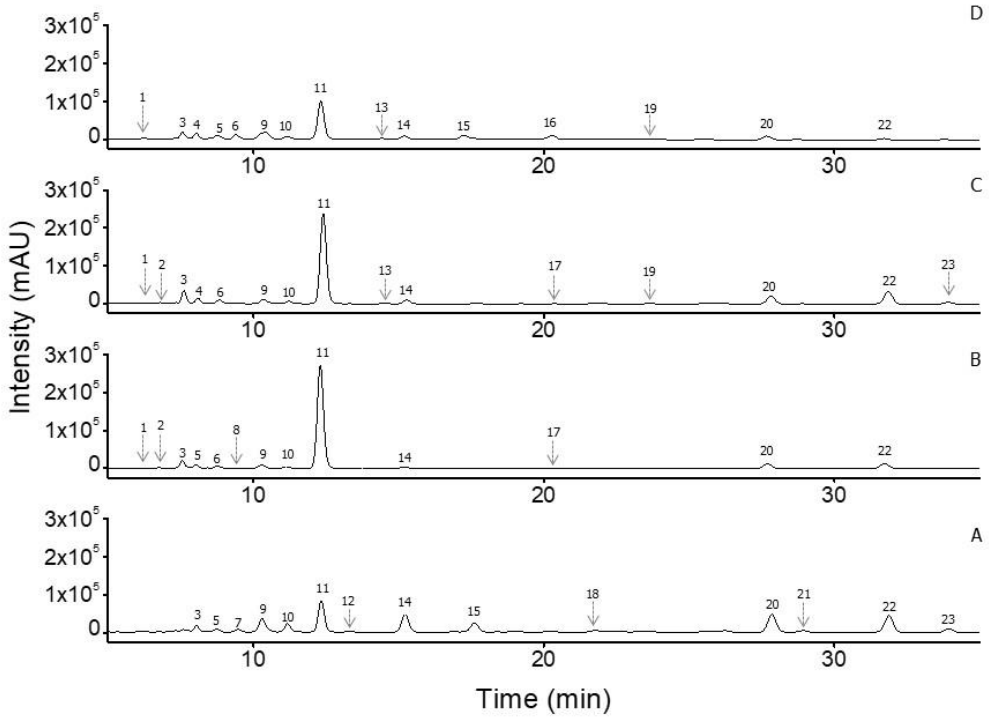


Figure 1. Chromatogram, obtained by HPLC-DAD, of carotenoid extract from *Chlorella vulgaris*. See text for chromatographic conditions, times 0h (A), 12h (B), 4h (C) and 96h (D). The identification and characterization of the peak are given in Table 1. The chromatogram was processed at 451 nm.

Table 3. Quantitative characterization of carotenoids in microalgal extracts of *Chlorella vulgaris* (μg/g dry weight).

Peak	Carotenoids	0h	4h	12h	96h
1	15-*cis*-neochrome	nd	nd	43.97±0.00	nd
2	13-*cis*-neoxanthin	nd	41.67±0.05	nd	nd
3	all-*trans*-neoxanthin	nd	43.34±0.14[a]	38.47±0.03[a]	nd
4	all-*trans*-neochrome	16.72±0.07[a]	106.97±0.08[b]	72.32±0.41[c]	nd
5	9-*cis*-neoxanthin	28.89±0.00[a]	69.59±0.38[b]	69.63±0.06[b]	23.90±8.23[a]
6	13-*cis*-antheraxanthin	nd	62.65±0.68[a]	65.43±0.09[a]	nd
7	all-*trans*-luteoxanthin	24.34±0.09	nd	nd	nd
8	all-*trans*-antheraxanthin	nd	40.42±0.30[a]	67.12±0.01[b]	12.88±6.51[c]
9	13-*cis*-lutein	45.24±0.08[a]	38.13±0.04[a]	73.68±0.16[b]	nd
10	15-*cis*-lutein	21.78±0.07[a]	16.97±0.08[a]	16.90±0.03[a]	3.48±0.12[b]
11	all-*trans*-lutein	96.68±0.39[a]	707.30±2.19[b]	308.97±0.27[c]	321.67±0.79[d]
12	13-*cis*-zeaxanthin	18.61±0.02	nd	nd	nd
13	all-*trans*-zeaxanthin	nd	43.43±0.73	nd	nd
14	9-*cis*-lutein	83.24±0.03[a]	72.93±0.24[b]	26.63±0.52[c]	nd
15	9-*cis*-zeaxanthin	53.01±0.60[a]	42.73±0.38[b]	67.82±0.19[c]	4.12±0.10[d]
16	2’-dehydrodeoxymyxol	nd	nd	nd	nd
17	5.6-epoxy-β-carotene	nd	nd	65.70±0.43	nd
18	all-*trans*-echinenone	nd	40.73±0.19	nd	nd
19	9-*cis*-echinenone	nd	44.19±0.19	nd	nd
20	all-*trans*-α-carotene	72.23±1.48[a]	81.51±1.89[b]	35.60±1.01[c]	16.41±1.10[d]
21	9-*cis*-α-carotene	6.87±0.40	nd	nd	nd

22	all-*trans*-β-carotene	92.82±2.56[a]	186.40±0.97[b]	50.94±0.24[c]	26.56±8.24[d]
23	9-*cis*-β-carotene	30.12±0.43[a]	58.07±2.46[b]	46.82±0.07[c]	nd

[1]Values are average and standard deviation of triplicates.
[2]Not detected.

The impact of microalgae growth kinetics on carotenoid composition can be seen in Figures 1, Table 2 and Table 3. In general, at time 0h, where the microalga was in the process of transition to the dark environment, the carotenoids total was 590.56μg.g^{-1}. Among the 13 carotenoids identified. The major carotenoids were all-trans-lutein (96.68 μg.g^{-1}, peak 11), all-trans-β-carotene (92.82μg.g^{-1}, peak 22), 9-cis-lutein (83.24μg.g^{-1}, peak 14) and all-trans-α-carotene (72.23μg.g^{-1}, peak 20) which corresponds to 59% of the total carotenoid content. Followed by 9-cis-zeaxanthin (53.01μg.g^{-1}, peak 15), 13-cis-lutein (45.24 μg.g^{-1}, peak 9), 9-cis-β-carotene (30.12μg.g^{-1}, peak 23), 9-cis-neoxanthin (28.89μg.g^{-1}, peak 5), all-trans-luteoxanthin (24.34μg.g^{-1}, peak 7), 15-cis-lutein (21.78μg.g^{-1}, peak 10), 13-cis-zeaxanthin (18.61μg.g^{-1}, peak 12), all-trans-neochrome (16.72μg.g^{-1}, peak 4) and 9-cis-α-carotene (6.87μg.g^{-1}, peak 21), have also been identified in this microalgae. Considering the compounds identified, the carotenoid profile agrees with morphological and cytological characteristics (Takaichi, 2020).

With increasing time, it is possible to observe in the first 4 hours significantly increased the total content of carotenoids by 1612,02μg.g^{-1}. With a total of 17 different carotenoids identified (Fig. 1), all-trans-lutein (707.30μg.g^{-1}) was the major carotenoid, followed by all-trans-β-carotene (186.40 μg.g^{-1}).

While 13-cis-neoxanthin (41.67μg.g^{-1}), all-trans-neoxanthin (43.34μg.g^{-1}), 13-cis-antheraxanthin (62.65μg.g^{-1}), all-trans-antheraxanthin (40.42μg.g^{-1}), all-trans-zeaxanthin (43.43μg.g^{-1}), all-trans-echinenone (40.73μg.g^{-1}) and 9-cis-echinenone (44.19μg.g^{-1}) were not detected in time 0 h but identified after 4 hours. Especially, it is worth noting that the total absence of light promoted the formation of all-trans-echinenone and 9-cis-echinenone, a microalgae carotenoid that is hugely revered due to its antioxidant potential provided by the conjugated double bonds present in its structure (Chang, Chang, & Lai, 2013; El-Gawad, Wang, & Yao, 2019; Papp et al., 2013; Rodrigues, Mariutti, Chisté, & Mercadante, 2012).

Compared to all the heterotrophic cell growth, the 4 hours differed significantly in terms of 24 hours, 48 hours, 72 hours, 96 hours and 120 hours. Where it was observed that the carotenoid content increased for most, except for 15-cis-lutein (peak 10).

Regarding the 8 hours, the total carotenoid content was 1085.85 $\mu g.g^{-1}$. Sixteen carotenoids were identified. All-trans-β-carotene (277.13$\mu g.g^{-1}$) along with all-trans-lutein (147.71$\mu g.g^{-1}$) were the majority components, which accounted for 39% of total carotenoids. Positively, 15-cis-neochrome (peak 1), all-trans-antheraxanthin (peak 8), all-trans-zeaxanthin (peak 13), dehydrodeoxymyxol (peak 16), all-trans-echinenone (peak 18) and 9-cis-echinenone (peak 19), which had not constituted the content in the 0 hours, were effectively identified into the 8 hours at concentrations ranging from 126.56 to 37.80$\mu g.g^{-1}$. Compared to all microalga growth times, the 8 hours and 12 hours differed significantly only of 96 hours.

The efficiency of production of total carotenoids from 16 hours was 1208,60$\mu g.g^{-1}$. When compared to different growth times the 16 hours differed significantly to 24 and 96 hours. The extract showed 18 carotenoids, the majority was all-trans-lutein (29.07%), followed by all-trans-β-carotene (15.95%). Additionally, unique microalgae carotenoids such as all-trans-echinenone (4.35%), 9-cis-echinenone (3.98%), and dehydrodeoxymyxol (3.47%) constituted the extract.

Finally, the 20 hours differed significantly in terms of 24 hours, 48 hours, 72 hours and 96 hours. The total content of carotenoids was 1194.74 $\mu g.g^{-1}$. All-trans-lutein and all-trans-β-carotene constituted altogether 26.36 and 24.04% of total carotenoids in the 20 hours.

The global lutein market was estimated at US$ 135 million in 2015 and will continue to rise until 2024 (Global Market Insights Lutein Market Report, 2016; Jacob-Lopes et al., 2019). Thus, the potential of microalgae seems undeniable, important issues will have to be solved, such as the industry inertia and the legal status of the carotenoids from microalgae, therefore heterotrophic cultivation of microalgae is a promising approach in facing the demand for natural lutein in the nutraceutical market.

4.Conclusion

The study by *Chlorella vulgaris* showed differences in relation to its qualitative profile of carotenoids in the different phases of growth. A total of 23 carotenoids were identified and the highest total carotenoid production was at 4h with 1612,02 $\mu g/g^{-1}$ (dry weight), and all-trans-lutein carotenoid majority. From this work, it is safe to recommend that species of the

genus *Chlorella* are suitable candidates for the heterotrophic production of lutein and demonstrate that carotenoids are also synthesized in the dark and heterotrophic culture seems to be an interesting option for the commercial production of these compounds. The focus on these organisms and the use of cultivation strategies and genetic engineering of processes can overcome the difficulties encountered in these crops for the production of natural pigments.

5. Acknowledgements

The authors are grateful to the National Academic Cooperation Program PROCAD/CAPES and National Counsel of Technological and Scientific Development (552440/2011-6 and 405640/2013-7) (CNPq) for the financial support. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

6.References

Aimjongjun, S., Sutheerawattananonda, M., Limpeanchob, N. (2013). Silk lutein extract and its combination with vitamin E reduce UVB-mediated oxidative damage to retinal pigment epithelial cells. Journal of Photochemistry and Photobiology A: Chemistry. B, 124, 34-41.

Alagoz, Y., Nayak, P., Dhami, N., Cazzonelli, C.I. (2018). cis-carotene biosynthesis, evolution and regulation in plants: The emergence of novel signaling metabolites. Archives of Biochemistry and Biophysics. 654, 172-184.

Behrens, P.W. Photobioreactors and fermentors: the light and dark sides of growing algae R.A. Andersen (Ed.), Algal Culturing Techniques, Elsevier, Oxford (2005), pp. 189-204.

Britton, G. (1995). UV/visible spectroscopy. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), Carotenoids: Spectroscopy (pp. 13–62). Badel: Birkhäuser Verlag.

Bukowski, M. R., Voeller, K., & Jahns, L. (2018). Simple and sensitive dilute-and-shoot analysis of carotenoids in human plasma. Journal of Chromatography B, 1095, 32–38.

Canelli, G., Tevere, S., Jaquenod, L., Dionisi, F., Rohfritsch, Z., Bolten, C. J., ... & Mathys, A. (2022). A novel strategy to simultaneously enhance bioaccessible lipids and antioxidants in hetero/mixotrophic *Chlorella vulgaris* as functional ingredient. Bioresource Technology, 126744.

Cha, K. H., Lee, H. J., Koo, S. Y., Song, D.-G., Lee, D.-U., & Pan, C.-H. (2010). Optimization of Pressurized Liquid Extraction of Carotenoids and Chlorophylls from *Chlorella vulgaris*. Journal of Agricultural and Food Chemistry, 58(2), 793–797.

Chacón-Lee, T.L., González-Mariño, G.E. (2010). Microalgae for "Healthy" Foods Possibilities and Challenges. Comprehensive reviews in food science and food safety, 9, 655-675.

Chung, R.W.S., Leanderson, P., Lundberg, A.K., Jonasson, L. (2017). Lutein exerts anti-inflammatory effects in patients with coronary artery disease. Atherosclerosis, 262, 87-9

De Rosso, V.V., & Mercadante, A.Z. (2007). Identification and quantification of carotenoids,by HPLC-PDA-MS/MS, from Amazonian fruits. Journal of Agricultural and Food Chemistry, 55, 5062–5072.

De Rosso, V.V., Mercadante, A.Z. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. Journal of Agricultural and Food Chemistry, 55 (2007), pp. 5062-5072.

Eisenreich, W., Bacher, A., Arigoni, D., Rohdich, F. (2004). Biosynthesis of isoprenoids via the non-mevalonate pathway. Cellular and Molecular Life Sciences; 61: 1401–1426.

Fernández-Sevilla, J.M.J.M., Acién Fernández, F.G., Molina Grima, E. (2010). Biotechnological production of lutein and its applications Applied Microbiology and Biotechnology., 86, 27-40.

Francisco, É. C., Franco, T. T., Wagner, R., & Jacob-Lopes, E. (2014). Assessment of different carbohydrates as exogenous carbon source in cultivation of cyanobacteria. Bioprocess and Biosystems Engineering, 37(8), 1497–1505.

Fraser, P.D., Schuch, W., Bramley, P.M. (2000). Phytoene synthase from tomato (Lycopersicon esculentum) chloroplasts-partial purification and biochemical properties. *Planta* 211, 361–369.

Global Market Insights Lutein Market Report. https://www.gminsights.com/industry-analysis/lutein-market (2016–2024).

Hilgarth, C., Sauer, N., Tanner, W. Glucose increases the expression of the ATP/ADP translocator and the glyceraldehyde-3-phosphate dehydrogenase genes in *Chlorella J*. Biol. Chem., 266 (1991), pp. 24044-24047.

Hodgson, P., Barrow, C., Adholeya, A. (2016). A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. Frontiers in Microbiology, 7, 1-19.

Hu, J., Nagarajan, D., Zhang, Q., Chang, J.-S., & Lee, D.-J. (2018). Heterotrophic cultivation of microalgae for pigment production: A review. Biotechnology Advances, 36(1), 54–67.

Huang, J. J., Lin, S., Xu, W., & Cheung, P. C. K. (2017). Occurrence and biosynthesis of carotenoids in phytoplankton. Biotechnology Advances, 35(5), 597–618.

Ip, P.-F., & Chen, F. (2005). Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. Process Biochemistry, *40(2), 733–738.*

Jacob-Lopes, E., Maroneze, M.M., Deprá, M.C., Sartori, R.B., Dias, R.R., Zepka, L.Q. (2019). Bioactive food compounds from microalgae: An innovative framework on industrial biorefineries, 25, 1-7.

Kitada, K., Machmudah, S., Sasaki, M., Goto, M., Nakashima, Y., Kumamoto, S., & Hasegawa, T. (2009). Supercritical CO2extraction of pigment components with pharmaceutical importance from *Chlorella vulgaris*. Journal of Chemical Technology & Biotechnology, 84(5), 657–661.

Komor, E., Schwab,W.G.W., Tanner, W. The effect of intracellular pH on the role of hexose uptake in *Chlorella* Biochem. Biophys. Acta, 555 (1979), pp. 524-530.

Komor,E., Tanner, T. The determination of the membrane potential of *Chlorella vulgaris*: evidence for electrogenic sugar transport Eur. J. Biochem., 70 (1976), pp. 197-204.

Krinsky,N.I., Landrum, J.I., Bone, R.A. (2003). Biological mechanisms of the protective role of lutein and zeaxanthin in the eye. Annual Review of Nutrition., 23, 171-201

Lin, J.H., Lee, D.J., Chang, J.S. (2015). Lutein production from biomass: marigold flowers versus microalgae. Bioresource Technology., 184, 421-428.

Liu, J., Sun, Z., Zhong, Y., Gerken, H., Huang, J., Chen, F. (2013). Utilization of cane molasses towards cost-saving astaxanthin production by a *Chlorella zofingiensis* mutante. Journal of Applied Phycology., 25, 447-1456.

Liu, J., Huang, J., Jiang, Y., Chen, F. (2012). Molasses-based growth and production of oil and astaxanthin by *Chlorella zofingiensis*. Bioresource Technology., 107, 393-398.

Maass, D., Arango, J., Wust, F., Beyer, P., Welsch, R. (2009). Carotenoid Crystal Formation in Arabidopsis and Carrot Roots Caused by Increased Phytoene Synthase Protein Levels. PLoS ONE 4(7): e6373.

Mapelli-Brahm, P., Corte-Real, J., Meléndez-Matinéz, A.J., Bohn, T. (2017). Bioaccessibility of phytoene and phytofluene is superior to other carotenoids from selected fruit and vegetable juices. Food Chemistry, 229, 304-311.

Mócsai, R., Figl, R., Troschl, C., Strasser, R., Svehla, E., Windwarder, M., Thader. A., Altmann, F. (2019). N-glycans of the microalga *Chlorella vulgaris* are of the oligomannosidic type but highly methylated. Nature Scientific Reports, 9(1).

Molazadeh, M., Danesh, S., Ahmadzadeh, H., & Pourianfar, H. R. (2019). Influence of CO2 concentration and N:P ratio on *Chlorella vulgaris*-assisted nutrient bioremediation, CO2 biofixation and biomass production in a lagoon treatment plant. Journal of the Taiwan Institute of Chemical Engineers. *In press.*

Panova, I.G., Yakovleva, M.A., Tatikolov, A.S., Kononikhin, A.S., Feldman, T.B., Poltavtseva, R.A., Nikolaev, E.N., Sukhikh, G.T., Ostrovsky, M.A. (2017). Lutein and its oxidized forms in eye structures throughout prenatal human development. Experimental Eye Research., 160, 31-37.

Panova, I.G., Yakovleva, M.A., Tatikolov, A.S., Kononikhin, A.S., Feldman, T.B., Poltavtseva, R.A., Nikolaev, E.N., Sukhikh, G.T., Ostrovsky, M.A. (2017). Lutein and its oxidized forms in eye structures throughout prenatal human development. Experimental Eye Research., 160, 31-37.

Patias, L.D., Fernandes, A. S., Petry, F.C., Mercadante, A.Z., Jacob-Lopes, E., Zepka, L.Q. (2017). Carotenoid profile of three microalgae/cyanobacteria species with peroxyl radical scavenger capacity. Food Research International, 100, 260-266.

Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y (2011) Heterotrophic cultures of microalgae: metabolism and potential products. Water Res 45:11–36.

Queiroz MI, Hornes MO, Silva-Manetti AG, Jacob-Lopes E (2011) Single-cell oil production by cyanobacterium Aphanothece microscopica Nägeli cultivated heterotrophically in fish processing wastewater. Appl Energy 88:3438–3443.

Rippka R, Derueles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61.

Rizwan M, Mujtaba G, Memon SA, Lee K, Rashid N. (2018) Exploring the potential of microalgae for new biotechnology applications and beyond: A review. Renewable Sustainable Energy Reviews, 92, 394-404.

Rodrigues, D.B., Menezes, C.R., Mercadante, A., Jacob-Lopes, E., Zepka, L.Q. Bioactive pigments from microalgae Phormidium autumnale. Food Research International, 77 (2015), pp. 273-279

Rodrigues, D.B., Flores, E.M.M., Barin, J.S., Mercadante, A.Z., Jacob-Lopes, E., Zepka, L.Q. From waste to natural pigments: Productions of microalgal carotenoids in agroindustrial wastewater Food Research International, 65 (2014), pp. 144-148.

Santos, A. M., Vieira, K. R., R. B., Santos, A. M., Queiroz, M. I., Zepka, L. Q., Jacob-Lopes, E. (2017). Heterotrophic Cultivation of Cyanobacteria: Study of Effect of Exogenous Sources of Organic Carbon, Absolute Amount of Nutrients, and Stirring Speed on Biomass and Lipid Productivity. Frontiers in Bioengineering and Biotechnology, 5.

Sauer, N., Tanner, W. The hexose carrier from *Chlorella.* cDNA cloning of a eukaryotic H^+-cotransporter FEBS Lett., 259 (1989), pp. 43-46.

Shi, X. –M., Zhang, X. –W., Chen, F. (2000). Heterotrophic production of biomass and lutein by *Chlorella* protothecoides on various nitrogen sources. Enzyme and Microbial Technology., 27, 312-318.

Sun, Z.; Li, T.; Zhou, Z.; Jiang, Y. (2015). Microalgae Biotechnology. (2th Ed.), Switzerland.

Takaichi. S. (2011). Carotenoids in algae: Distributions, biosyntheses and functions. Marine Drugs, 9 (6), 1101-1118

Tanner, W. (1969). Light-driven active uptake of 3-O-methylglucose via an inducible hexose uptake system of *Chlorella*. Biochemical and Biophysical Research Communications, 36(2), 278–283.

Tijani, H., Yuzir, A., Dagang, W. R. Z. W., Zamyadi, A., & Abdullah, N. (2018). Multi-parametric modelling and kinetic sensitivity of microalgal cells. Algal Research, 32, 259–269.

Tkáčová, J., Klempová, T., & Čertík, M. (2017). Kinetic study of growth, lipid and carotenoid formation in β-carotene producing Rhodotorula glutinis . Chemical Papers, 72(5), 1193–1203.

Van Breemen, R.B., Dong, L., & Pajkovic, N.D. (2012). Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids. International Journal of Mass Spectrometry, 312, 163–172.

Van Breemen, R.B., Dong, L., Pajkovic, N.D. Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids. International Journal of Mass Spectrometry, 312 (2012), pp. 163-172.

Wang, Y., Peng, J. (2008). Growth-associated biosynthesis of astaxanthin in heterotrophic *Chlorella zofingiensis* (Chlorophyta). World Journal of Microbiology and Biotechnology., 24, 1915-1922.

Wen, Z. Y., and Chen, F. (2003). Heterotrophic production of ecosapentaenoic acid by microalgae. *Biotechnol. Adv.* 21, 273–294.

Wu, Z.Y., Qu, C.B., Shi, X.M. (2009). Biochemical system analysis of lutein production by heterotrophic *Chlorella pyrenoidosa* in a fermentor. Food Technology and Biotechnology, 47, 450-455

Yang, C., Hua,Q., Shimizu, K. (2000). Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions. Biochemical Engineering Journal . 6, 87-102

Yeh, T.J., Tseng, Y.F., Chen, Y.C., Hsiao, Y., Lee, P.C., Chen, T.J., Chen, C.Y., Kao,C.Y., Chang, J.S., Chen, J.C., Lee, T.M. (2017). Transcriptome and physiological analysis of a lutein-producing alga Desmodesmus sp. reveals the molecular mechanisms for high lutein productivity. Algal Research., 21, 103-119

ANEXOS

CAPÍTULO 3

CAPÍTULO: Bioconversion of industrial wastes into biodiesel feedstocks

Capítulo publicado no livro “Sustainable Bioconversion of Waste to Value Added Products” (ISBN: 978-3-030-61837-7), publicado pela Springer Nature

CAPÍTULO 4

CAPÍTULO: Microalgae application in chemicals enzymes, and bioative molecules

Capítulo publicado no livro “Application of Microbes in Environmental and Microbial Biotechnology” (ISSN 2662-1681), publicado pela Springer Nature

Chapter Proposal

Bioconversion of Industrial Wastes into Biodiesel Feedstocks

Rosangela Rodrigues Dias, Mariana Manzoni Maroneze, Álisson Santos de Oliveira, Patrícia Acosta Caetano, Leila Queiroz Zepka and Eduardo Jacob-Lopes

Department of Food Science and Technology, Federal University of Santa Maria (UFSM), Roraima Avenue, 1000, 97105-900, Santa Maria, RS, Brazil
E-mail address: ejacoblopes@gmail.com

Abstract

To date, it has not been possible to establish the economic viability of the production of microalgae biodiesel. Relevant issues associated with commercial-scale microalgae cultivation need to be addressed to make this biofuel a reality. The high demand for water and nutrients represents a significant challenge. The use of wastewater for bioenergy production is an economically and environmentally promising alternative. In this context, the main objective of this chapter is to present a landscape of the potential use of microalgae for bioconversion of industrial wastes into biodiesel feedstocks. Initially, the microalgae will be presented as an auspicious feedstock for biodiesel. The use of industrial waste as a nutrients font for microalgae culture and biodiesel production will be discussed. The challenges associated with the bioconversion of industrial waste into biodiesel will be debated in its main aspects. In the end, the biodiesel characteristics and the economic issues of the commercialization of microalgae biodiesel from waste will be addressed.

Keywords: Microalgae, Wastewater; Lipids, Biofuel.

1 Introduction

Based on the current biotechnological maturity, unfortunately, it has not yet been possible to establish the economic viability of microalgae biofuels. The technological routes are immature, and the production cost makes it difficult to use microalgae as a producer of bioenergy [1]. However, although it has a high production cost, there is a global effort to make microalgae technology commercially attractive. Today, large and

medium-sized companies are investing in research and development to produce microalgae biofuels on a commercial scale. This attempt is supported by the initiative of many companies, such as Euglena, BP plc, and ExxonMobil [2].

A viable solution to reduce production costs is cultivation using wastewater. Scientists worldwide have demonstrated the important role of microalgae in bioremediation and nutrient recovery from wastewater [3,4]. The wastewater is a readily available source of water and nutrients for biomass production, which can be utilized to produce biodiesel [5,6]. The demand for biodiesel is increasing worldwide not only by the urgency to minimize dependence on fossil fuels, but also to maintain the sustainability of the ecosystem [7].

Until the moment, among renewable bioenergy sources, the microalgae have shown the most promise for the biodiesel production. Concomitant, an increasing number of studies have demonstrated the potential for bioconversion of municipal, agricultural, industrial, and agro-industrial waste into bioenergy. The strategy can considerably improve the sustainability of the production chain. It is predicted that, with the advance of research, the production expenses will decrease considerably, leading to the commercial success of the microalgae biodiesel companies.

Today, great emphasis has been given to the massive generation of industrial and agro-industrial waste, such as flue gases and wastewater. The wastewater from these sources has an expressive content of organic matter and are being evaluated for the microalgae cultivation and biodiesel production [8]. Given the potential use of these wastes for the economic viability of microalgae biodiesel production, the main objective of this chapter is to present a landscape of the use of microalgae for bioconversion of industrial wastes into biodiesel feedstocks.

2 Microalgae as a biodiesel feedstock

The overexploitation of energetic natural resources has driven the research and development (R&D) sector to seek alternative sources of energy to supply the growing demand and reduce the dependence on fossil fuels. Environmentally friendly fuels that do not harm human health and the ecosystems are in the focus of researchers around the world [9]. In particular, the biodiesel with properties similar to diesel, a non-renewable fuel widely used by trucks, buses and tractors and other machines that require high power, is gaining more and more space. It is worth highlight that among the advantages of

biodiesel using is the significant reduction in the emission of polluting gases, providing thus a high environmental gain [10].

The substitution of non-renewable fuels by renewable ones reflects helpful contributions to the economy and preservation of the environment. As sustainable and renewable alternative sources of energy have been full-blown the biofuels of the first, second, third, and fourth generation. The biodiesel generated from the oilseeds is named of first-generation; generated from the non-edible inputs of second-generation and generated from the organisms with elevated lipid synthesis of third-generation. The fourth-generation biodiesel production use microorganisms genetically modified and is an emerging approach [11].

Currently, biodiesel produced from food sources, through the exploitation of vegetable oils and others that are from an edible source, is already being applied directly to diesel engines, or in parallel with fossil diesel [12]. However, the use of arable lands is one of the disadvantages of using these sources. With the current concerns of international agencies, related to hunger and a significant growing in the price of foods, new sources have been explored, as is the example of oleaginous microorganisms, among them microalgae. These microorganisms have high synthesis and lipid storage in their cells [13].

The benefit of biodiesel fabrication from microalgae include the fast growth of cultures, high oil productivity, and utilize of non-arable land [14]. As potential biological agents, these microorganisms can valorize wasted resources, mitigate carbon dioxide (CO_2), and the biomass generated can be utilized for biodiesel production [15].

3 Low-cost waste as feedstock for biodiesel

The use of alternative fuels to oil products is promising in reducing the negative environmental impacts caused by the consumption of fossil fuels. The excessive use of these causes an increase in greenhouse gas emissions; thus, the focus of the research has become the development of renewable and environmentally friendly technologies that serve as a commercially available energy source [16].

Biodiesel is a promising biofuel to replace diesel [17]. The use of oleaginous microorganisms is an option for biodiesel production. It offers advantages due to its short cultivation period, higher productivity, and similarity in the fatty acid composition with the vegetable oils generally used [18,19].

Oilseed microorganisms accumulate a high concentration of lipids in their cells, many times greater than 20%, and using organic and inorganic carbon sources; the metabolism is carried out [20,21]. Unfortunately, the cost of cultivating these microorganisms is very high, hampering the economic viability of microbial oils [22]. However, the commercial and sustainable production of this bioproduct can be carried out when cultivated in low-cost substrates, such as organic and inorganic waste [23].

Waste treatment is mediated by primary and secondary, where the removal of solids occurs with the subsequent bioremediation of organic and inorganic materials by microorganisms. Microalgae appear as an alternative approach to biological treatment and act as removers of organic and inorganic fillers with subsequent conversion to biomass, which can be exploited to obtain various bioproducts, such as biofuels [24].

According to Pittman et al. [25] and Lundquist et al. [26], only cases involving treatment of industrial effluents with subsequent production of biofuel can generate biodiesel at a competitive cost in the market; without this process, it is economically unfeasible and does not offer a positive return. Therefore, microalgae applied to wastewater with subsequent generation of biodiesel can be considered a sustainable and renewable production of bioproducts.

Based on a study with microalgae, Chisti [27] observed a regular life cycle and concluded that in 24 hours, the lipid capacity in the microalgae biomass varies between 20 and 50%, and with genetic engineering techniques, this time can still be reduced. Mathimani et al. [28], were successful in testing the biodiesel harvested from microalgae mixed with petroleum diesel, obtained a reduction in the emission of carbon monoxide and CO_2 from the engine.

The application of residues as a media of cultivation to decrease the high expense of producing microalgae has been an alternative option favorable. This type of process has similar or superior potential in the production of lipids in microorganisms, besides reducing charge mainly nitrogen and phosphorus of residues to treated, these substrates are ideal for the generation and development of algae lipids [23]. It is worth mentioning that besides residue's composition, the efficiency of each strain must be explored simultaneously, according to the number of nutrients available for the development and production of lipids [19].

There are several types of waste, including agricultural, industrial, and municipal wastewater, and each can offer a different lipid production potential [29]. According to

Table 1, it is possible to evaluate some types of waste and its composition favorable to the current production of lipids in microalgae to obtain biodiesel.

[Insert Table 1]

3.1 Industrial waste as a nutrients font for biodiesel production

In the last decades, there has been a massive generation of industrial and agro-industrial waste. These residues, such as wastewater and flue gases, have organic and inorganic compounds that are useful for the commercial cultivation of microalgae. The use of these wasted resources can generate high value and low added-value products such as biodiesel.

Typically, the textile industries produce a high amount of residual water; in them, several fabric dyes are found [39]. The textile industry's wastewater contains essential nutrients for the growth of microalgae; they are characterized by intense colors, high salinity, unstable pH, and high demand for chemical oxygen. The nutrients are converted by chemically and biochemically to lipid content, which reaches up to 85% of dry biomass, and later biodiesel production is used [40]. This percentage is a determining factor in the application of microalgae for the production of biodiesel since a large part is composed of neutral lipids, mainly triacylglycerides (TAG). The use of industrial textile waste as a biodiesel production process is a sustainable strategy that reduces large-scale damage to the wastewater receiving environment [2,31].

Regarding rubber production, carried out through the transformation of latex, is a large amount of wastewater is generated, such as washing water, protein whey, non-gelled latex, lipids, carbohydrates, salts, ammonia, nitrate, phosphorus, and total solids [8]. Few studies have been carried out with microalgae applied to industrial rubber waste since they are not produced in abundance in different regions. However, Bich et al. [41] and Ayyasamy et al. [42] reported that microalgae consumed the nutrients contained in the waste by up to 93.4%, with high biomass productivity and lipid biosynthesis that improve the production of biofuels [8].

The industrial sewage sludge contains nitrified compounds and inorganic pollutants such as cadmium, copper, lead, and selenium. According to Lim et al. [43], the use of microalgae combined with the symbiotic application with bacteria improves the denitrification process. Besides this, increases the performance of the microalgae with high lipid production. Torres et al. [33] concluded that the lipid content is not affected, the contaminants favored the increase of the biomass, demonstrating that the microalgae

integrated to activated sludge substrates for the simultaneous production of components for biodiesel, simultaneously support the environmental sector through waste treatment.

About pharmaceutical wastewater, they have a significant and diverse amount of organic compounds that can remain in aquatic environments and are persistent in degradation by microorganisms. However, the use of microalgae has become a sustainable and comprehensive strategy [44]. Combined with the subsequent extraction of microalgae oils to the biodiesel manufacturing from the biomass produced, a symbiotic system, microalgae, and bacteria can remove 60 to 90% of the contaminating compounds [45,46].

A large group of wastewater includes agro-industrial waste, such as swine wastewater, milk manure, sugarcane bagasse hydrolyzate, beer fermentation waste, effluents from palm oil mill among others [30,36,37,47]. A common aspect of this type of waste is the presence of a high concentration of ammonium and chemical oxygen demand (COD) (20,180 mg L^{-1}). Thus, the co-culture of microalgae in these residues is a potential solution [48]. The technology for reducing the nutrient load, and having a high accumulation of lipids, through the facility to tolerate stress, becomes an efficient means for biodiesel manufacturing [49].

Noteworthy, Chinnasamy et al. [50] reported that the application of algae in the industrial residues of a carpet factory could produce approximately 15,000 tons of microalgae biomass, with the production of up to 4 million liters of biodiesel, and removal of around 1500 tons of nitrogen and 50150 tons of phosphorus from this wastewater a year. Notably, the microalgae are promising for the biodiesel production with the cultivation in low-cost waste, such as industrial waste. The yield of lipid production and composition from microalgae has emerged as an attractive path in large-scale biodiesel production. Additional studies and explorations of the yield of each strain in different kinds of waste for the production of biodiesel can promote the commercialization of this biofuel [51].

4 Challenges of the bioconversion of industrial waste to biodiesel

The biodiesel production from microalgae involves upstream and downstream processing, which includes the unit operations of strain selection, cultivation, harvesting, drying, extraction and conversion techniques, as shown in Figure 1. Until today, the technology to transform microalgal biomass into biodiesel is technically feasible but uneconomical at commercial scale. The economic feasibility of any microalgae-based

process depends on the choices of methods for each unit operation in the upstream and downstream phases.

[Insert Figure 1]

The most significant challenges to improve the economic viability of any microalgae-based process are related to three main aspects: (i) improve cultivations productivity; (ii) reduce the energy demand for the downstream processing, especially for harvesting, drying, and oil extraction; and (iii) explore the full potential of microalgal biomass in a multi-product biorefinery concept [52]. The first challenge includes selecting the strain, finding a low-cost cultivation medium, prioritizing industrial wastes, and choosing the most suitable cultivation system. Downstream processing of microalgae biodiesel represents about 60% of the total production cost of the biofuel, so in the second approach, more economical and integrated techniques are required for the main steps of the process. The last issue refers to process design strategies that not only aim a single product but also to a whole valorization of all biomass fractions.

The first, and critical, step in microalgae-based processes for the biodiesel production, or any other product is the choice of the microalgae strain to be grown [53,54]. To date, there are more than 158,300 strains cataloged, according to algaebase.org, each with its characteristics and requirements. Due to this large number of species available, a robust selection is challenging due to the limited information on most of these microalgae and their distinct characteristics [55].

In general, the desired characteristics of microalgae strain for biodiesel production include rapid growth rate, high lipid content, grow over a wide range of temperature, salinity and irradiation (for photosynthetic cultures), high shear and oxygen tolerance, grow in a selective environment to reduce the possibility of contamination, ease of harvesting, weak cell wall, and suitable fatty acid profile [53,56]. Considering that the building blocks for biodiesel production are lipids and that they are intracellular, lipid productivity is typically considered as a decisive parameter in the choice of the strain, since it considers both the lipid content and the biomass productivity [57,58]. Additionally, when the culture medium is wastewater, the selected strain must have resistance to the nutrients present, especially ammoniacal nitrogen, which at high concentrations can become toxic and inhibit growth [59].

As for their origin, microalgae strains can either be obtained from stock culture collections or be isolated from environmental samples [60]. Still, it is difficult to find a strain that includes all the required properties. In this sense, one option to improve the

strains is to modify them by mutagenesis or genetic engineering techniques, including genome editing tools and metabolomic re-programming. The use of these techniques becomes increasingly crucial for the industrial viability of microalgal-based products, especially for low-value products such as biodiesel. This becomes even more imperative when using industrial wastes as a culture medium, as it requires greater robustness of the culture [61].

Once defined the strain, the cultivations aspects need to be addressed, especially the unresolved bottlenecks. The first point to consider is the cultivation mode and system. One of the advantages of microalgae is their metabolic versatility. Although the preferred route is photoautotrophic, these microorganisms can also assume other types of metabolisms, including heterotrophic and mixotrophic. Regarding cultivation systems, in industrial-scale, microalgae are usually cultured in open or closed systems [62].

Photoautotrophic cultivation refers to the process in which light energy is captured and, an inorganic source of carbon is used to form chemical energy through photosynthesis process. In this cultivation model, microalgae primarily require an inorganic carbon source, like CO_2, and light energy [14]. Since CO_2 can come from industrial waste and light energy can be supplied by sunlight, this type of process is considered environmentally friendly and has so far been the most widely used. In this type of cultivation, open raceway ponds are still the most adopted system to cultivate microalgae for industrial production of low-cost products, including biofuels, since these facilities are inexpensive and easy to operate than closed systems. On the downside, open systems have some operational problems as the dependence on climate conditions, contamination, evaporation, and extensive land requirements. Due to the high cost, closed photobioreactors are more suitable to be used to produce higher market value products like carotenoids and fatty acids. Besides this, the dependence on light energy restricts the scale-up and hinders the design of the cultivation systems [13,63].

A feasible alternative is the heterotrophic growth in the absence of light, supported by an exogenous carbon source, which can overcome the major limitations of autotrophic cultures. Although not all species can use respiratory metabolism, when possible, the heterotrophic cultures can be efficiently conducted in conventional fermenters, e.g., stirred tank and bubble column bioreactors, where, in general, are cheap, simple to construct, and easy to scale and maintain on a large scale [64,65]. On the other hand, the biggest challenge of heterotrophic cultures is the demand for exogenous organic carbon, since in these types of cultivation the carbon source represents about 80% of the cost of

the culture medium [6]. In this sense, the obtainment of organic carbon and other nutrients from industrial wastes may offer an inexpensive alternative for microalgae cultures, with parallel wastewater treatment [66].

Another option is the mixotrophic cultivation that is a variant of the heterotrophic growth regime. In this case, the microorganisms simultaneously assimilate organic carbon and CO_2 and use both photoautotrophy and heterotrophy [67]. Since photosynthesis is not the only route available for obtaining energy, microalgal growth is not strictly dependent on light. This eases the geometry of the photobioreactors, making the scaling-up easier. The differential of this mode of cultivation is that it is possible to use both wastewaters as culture medium and CO_2 from industrial wastes [68].

Regardless of the cultivation method, on a commercial scale, the algal cultures require an enormous amount of freshwater and compounds like carbon, nitrogen, phosphorous, and several other trace nutrients [69]. Thus, the production of microalgae-based products in an economical way depends on the source of water and nutrients used. As already discussed, industrial wastes are a source of nutrients useful to support the microalgae growth, nonetheless still has some bottlenecks that need to be considered. The main setbacks are the possibility of the presence of biotic or abiotic growth inhibitors and complicated harvesting processes. These issues will depend on the source of wastewater, and for this reason, they must be washed into consideration when choosing the waste for biotechnological use [59].

The biotic factors can be present in the form of viruses, fungi, bacteria, zooplankton, and predators. Once established, herbivorous consumers can reduce or inhibit the microalgae growth within just a few days. Besides, the contamination with fungi and viruses can negatively affect microalgal growth and induce changes in microalgal cell arrangement, diversity, and succession [70]. To overcome these biological barriers, the integrated pest management that involves the application of chemical herbicides and pesticides has been identified as a viable solution, on the other hand, it will result in an augmentation in the costs of the process, and with the prolonged use, the microbiota may acquire resistance to these substances [71]. Other options with great potential for success include ecological engineering strategies of aquatic communities to promote beneficial interactions and genetic and metabolic engineering techniques to improve the resistance of the microalgae strains [72].

The abiotic contaminants that can be present in wastewater include heavy metals, nitrogen oxides, sulfur oxides, and ammonia, which in high concentrations can inhibit

microalgae growth. Clijsters and Assche [73] demonstrated that in the presence of several heavy metals, the chloroplast ultra-structure was disorganized. Besides this, these compounds can inhibit microalgae photosynthesis at physiological levels by blocking the prosthetic metal atoms in the active site of important enzymes [74]. At the same time, when concentrations of essential nutrients in wastewater are low, they need to be supplemented so that there is no reduction in growth rates and lipid productivity [59].

5 Biodiesel characteristics

The biodiesel properties depend on the fatty acid profile of the feedstocks used, which may vary from one another. Table 2 shows the fatty acid profile of distinct biodiesel feedstocks. The biodiesel from the different feedstocks must meet a specifications series. The biodiesel properties established by ASTM International (ASTM D6751), European Union (EN 14214), and Brazil (ANP 45) are shown in Table 3 [75-77].

[Insert Table 2]

[Insert Table 3]

The biodiesel quality is influenced by the fatty acid profile, contaminants presence of the feedstock, production process and storage. The properties of biodiesel related to fatty acid profile and contaminants inherent to the feedstock include the iodine value, viscosity, cloud point, cetane number and phosphorus content. On the other hand, the properties of biodiesel directly related to the production process include free and total glycerin, carbon residue, ester content, methanol content and flashpoint, while those related to storage include oxidative stability, acidity value and content of water [10,80].

As shown in Table 3, the biodiesel from microalgae biomass grown in agro-industrial wastewater has an ester content of about 99%, a cetane number of 55, an iodine value of $73.5gl^{2}100g^{-1}$, and a degree of unsaturation of 75% [79]. Noteworthy, the microalgae appear to be the most realistic biodiesel feedstock, capable of replacing traditional fuels in way more environmentally friendly. These microorganisms can be capable of compensating and balance the growing demands for bioenergy [14].

Many countries of Europe and America have begun to assess the possible commercialization of biofuels from the microalgae biomass. Many microalgae are favorable to the production of biofuel due to the high content of lipids. The current unfeasibility of microalgae biodiesel is due to the elevated production cost [81]. However,

this cost can be reduced considerably with the use of industrial residues as a source of nutrients and water for cultivation [82].

Besides, one of the advantages of utilizing microalgae is that they can grow from distinct routes, such as photoautotrophic, heterotrophic, and mixotrophic. Through these routes, different sources of organic and inorganic carbon are assimilated. It is worth mentioning that it is crucial to select the most suitable strain for oil production for biodiesel. Typically, microalgae have an oil content between 20 and 50% of dry weight but can reach 70% [81,83].

6 Economic aspects of the commercialization of microalgae biodiesel from waste

The world is facing an energy crisis due to the progress of industrialization and the high exploitation and depletion of natural resources, such as fossil fuels. These represent about 88% of the total energy consumption [84,85]. Biofuels are a promising new source of energy; they are renewable and can be obtained through existing biological resources [19].

Microalgae-based biodiesel and its economic viability have received extensive academic exploration [86,87]. To be considered a viable substitute for fossil fuels, the production of microalgae biodiesel needs to have its high cost reduced, which may be possible through technological and management innovations. Microalgae stand out for growing without the need for considerable territorial space and end up not competing with other food crops for land use [88].

The economic viability of biodiesel production systems, combined with the use of effluents, unfortunately, is not widely discussed on a pilot and commercial scale. The vast majority of techno-economic studies focus on photobioreactor or open lagoon processes. However, to fully understand the performance of wastewater use, some factors must be considered, such as reducing waste treatment costs, selling other generated by-products, such as bio-oil, biogas and biofertilizers [89].

According to the report by Grand View Research Inc., (2017), by 2025, the world market for biofuels for microalgae is expected to reach US$ 10.73 billion, with an 8.8% growth rate due to research by alternative sources of products to replace fossil fuels. Microalgae biodiesel has a 20 times higher yield than plant derivatives, serving as an environmentally friendly source of biofuels [90]. Namely, three criteria are essential when evaluating a process for the biofuels industry: energetic, economical, and mainly

environmental and sustainability [91]. According to Figure 2, we can see the price of classic products manufactured through traditional refinements and biorefineries.

[Insert Figure 2]

According to the monthly report of the International Energy Agency (IEA), the value of the oil barrel is around US$ 27 to 34, varying according to the exporting country [93]. Oil diesel and gasoline are fuels with higher demand and global production; relatively low prices are quoted by these, between US$ 0.85 and 0.75/L [13].

With the discoveries of oil exploration sources, natural gas, pre-salt, shale gas, there is a reduction in the availability of crude oil, an increase in prices, and expansion in search of substitutes [95]. With this, explorations in biorefineries appear, serving as a circular economy for biodiesel production through edible oils was produced. However, the cultures of edible vegetable oil, besides requiring large tracts of land, could cause an economic crisis in the food market due to rising prices; besides this, the production cost is up to 3 times higher than traditional diesel [1].

According to Chen et al. [92], the biodiesel cost based on microalgae production is from US$ 0.42 to 22.60/L. Based on a techno-economic study, Xin et al. [89], evaluated that obtaining biofuel from microalgae produced with wastewater is considered favorable, making the cost of this bioproduct competitive with that of oil and more imminent to the commercial reality.

According to Ventura et al. [96], the use of industrial wastewater in the microalgae cultivation reduces about 30% of the total operating cost, reducing the cost of nutrients for biomass production in US$ 550,000/year. To improve economic viability, higher biomass productivity and the lipid composition of microalgae must be explored for more considerable expansion in biodiesel production. Concerning capital investments for microalgae biodiesel, these are still high.

Still, the primary contrast between the commercialization of algae biodiesel and vegetable oil biodiesel is sustainability. For planting lipid-producing seeds are needed many hectares and large amounts of water for irrigation. On the other hand, the microalgae application in industrial waste has emerged as an attractive service scale. It results in low-cost production and lipid yield for the large-scale production of superior biodiesel, can being able to generate other bioproducts with high added value, making it more realistic - the biodiesel industrialization [97,98].

7 Concluding remarks

Microalgae are widely hailed as one of the most sustainable resources for biodiesel production. Nevertheless, the economic viability of the industrial production of microalgae biomass is still in shadows of doubt. In this sense, the use of industrial wastes is an option to upgrade the economic sustainability of the bioprocess, besides contributing to the environmental management of wastes. Even so, we still are on the way to improve this futuristic idea and raise it to a commercial level, since there are still technical and economic bottlenecks to be solved. In order to consolidate the third generation microalgae biofuel industry, in addition to the use of industrial waste, other strategies are also crucial to achieving this goal, including: (i) harness the full potential of biomass, through the use of a biorefinery approach; (ii) use of genetic and metabolic engineering to improve the microalgae strains resistance and the yields of the bioproducts of interest; and (iii) improve downstream processing techniques, aiming at integrated and more economical processes.

References

[1] Deprá, M. C., dos Santos, A. M., Severo, I. A., Santos, A. B., Zepka, L. Q., Jacob-Lopes, E. (2018). Microalgal biorefineries for bioenergy production: can we move from concept to industrial reality?. BioEnergy Research, 11(4), 727-747.

[2] Salama, E. S., Kurade, M. B., Abou-Shanab, R. A., El-Dalatony, M. M., Yang, I. S., Min, B., Jeon, B. H. (2017). Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renewable and Sustainable Energy Reviews, 79, 1189-1211.

[3] Mondal, M., Halder, G., Oinam, G., Indrama, T., Tiwari, O. N. (2019). Bioremediation of organic and inorganic pollutants using microalgae. In New and Future Developments in Microbial Biotechnology and Bioengineering (pp. 223-235). Elsevier.

[4] Queiroz, M. I., Hornes, M. O., da Silva Manetti, A. G., Zepka, L. Q., Jacob-Lopes, E. (2013). Fish processing wastewater as a platform of the microalgal biorefineries. Biosystems Engineering, 115(2), 195-202.

[5] Paniagua-Michel, J. (2015). Bioremediation with Microalgae: Toward Sustainable Production of Biofuels. In Handbook of Marine Microalgae (pp. 471-481). Academic Press.

[6] Francisco, É. C., Franco, T. T., Zepka, L. Q., Jacob-Lopes, E. (2015). From waste-to-energy: the process integration and intensification for bulk oil and biodiesel production by microalgae. Journal of Environmental Chemical Engineering, 3(1), 482-487.

[7] Jayakumar, S., Yusoff, M. M., Rahim, M. H. A., Maniam, G. P., Govindan, N. (2017). The prospect of microalgal biodiesel using agro-industrial and industrial wastes in Malaysia. Renewable and Sustainable Energy Reviews, 72, 33-47.

[8] Udaiyappan, A. F. M., Hasan, H. A., Takriff, M. S., Abdullah, S. R. S. (2017). A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. Journal of Water Process Engineering, 20, 8-21.

[9] Ingrao, C., Bacenetti, J., Bezama, A., Blok, V., Goglio, P., Koukios, E. G., ... Huisingh, D. (2018). The potential roles of bio-economy in the transition to equitable, sustainable, post fossil-carbon societies: Findings from this virtual special issue. Journal of cleaner production, 204, 471-488.

[10] Cavalheiro, L. F., Misutsu, M. Y., Rial, R. C., Viana, L. H., Oliveira, L. C. S. (2020). Characterization of residues and evaluation of the physico chemical properties of soybean biodiesel and biodiesel: Diesel blends in different storage conditions. Renewable Energy, 151, 454-462.

[11] Kumar, R., Ghosh, A. K., Pal, P. (2020). Synergy of biofuel production with waste remediation along with value-added co-products recovery through microalgae cultivation: A review of membrane-integrated green approach. Science of the Total Environment, 698, 134169.

[12] Ayoola, A. A., Hymore, F. K., Omonhinmin, C. A., Olawole, O. C., Fayomi, O. S. I., Babatunde, D., Fagbiele, O. (2019). Analysis of waste groundnut oil biodiesel production using response surface methodology and artificial neural network. Chemical Data Collections, 22, 100238.

[13] Severo, I. A., Siqueira, S. F., Deprá, M. C., Maroneze, M. M., Zepka, L. Q., Jacob-Lopes, E. (2019). Biodiesel facilities: What can we address to make biorefineries commercially competitive?. Renewable and Sustainable Energy Reviews, 112, 686-705.

[14] Maroneze, M. M., Deprá, M. C., Zepka, L. Q., Jacob-Lopes, E. (2019). Artificial lighting strategies in photobioreactors for bioenergy production by *Scenedesmus obliquus* CPCC05. SN Applied Sciences, 1(12), 1695.

[15] Patidar, S. K., Mishra, S. (2017). Carbon sequestration by microalgae: a green approach for climate change mitigation. Encyclopedia of Sustainable Technologies, 477-483.

[16] Rajaeifar, M. A., Akram, A., Ghobadian, B., Rafiee, S., Heijungs, R., Tabatabaei, M. (2016). Environmental impact assessment of olive pomace oil biodiesel production and consumption: A comparative life cycle. Energy, 106, 87-102.

[17] Oh, P. P., Lau, H. L. N., Chen, J., Chong, M. F., Choo, Y. M. (2012). A review on conventional technologies and emerging process intensification (PI) methods for biodiesel production. Renewable and Sustainable Energy Reviews, 16, 5131and 45.

[18] Alptekin, E. (2017). Emission, injection and combustion characteristics of biodiesel and oxygenated fuel blends in a common rail diesel engine. Energy, 119, 44–52.

[19] Cho, H. U., Park, J. M. (2018). Biodiesel production by various oleaginous microorganisms from organic wastes. Bioresource Technology, 256, 502–508.

[20] Xu, X., Kim, J. Y., Cho, H. U., Park, H. R., Park, J. M. (2015). Bioconversion of volatile fatty acids from macroalgae fermentation into microbial lipids by oleaginous yeast. Chemical Engineering Journal, 264, 735-743.

[21] Amara, S., Seghezzi, N., Otani, H., Diaz-Salazar, C., Liu, J., Eltis, L. D. (2016). Characterization of key triacylglycerol biosynthesis processes in rhodococci. Scientific reports, 6(1), 1-13.

[22] Cho, H. U., Kim, Y. M., Choi, Y., Xu, X., Shin, D. Y., Park, J. M. (2015). Effects of pH control and concentration on microbial oil production from *Chlorella vulgaris* cultivated in the effluent of a low-cost organic waste fermentation system producing volatile fatty acids. Bioresource Technology, 184, 245-250.

[23] Cho, H. U., Kim, Y. M., Park, J. M. (2017). Enhanced microalgal biomass and lipid production from a consortium of indigenous microalgae and bacteria present in municipal wastewater under gradually mixotrophic culture conditions. Bioresource Technology, 228, 290-297

[24] Mohd Udaiyappan, A. F., Abu Hasan, H., Takriff, M. S., Sheikh Abdullah, S. R. (2017). A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. Journal of Water Process Engineering, 20, 8–21.

[25] Pittman, J. K., Dean, A. P., Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. Bioresource Technology, 102(1), 17–25.

[26] Lundquist, T. J., Woertz, I. C., Quinn, N. W. T., Benemann, J. R. (2010). A realistic technology and engineering assessment of algae biofuel production. Energy Biosciences Institute, 1.

[27] Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology advances, 25(3), 294-306.

[28] Mathimani, T., Senthil Kumar, T., Chandrasekar, M., Uma, L., Prabaharan, D. (2017). Assessment of fuel properties, engine performance and emission characteristics of outdoor grown marine *Chlorella vulgaris* BDUG 91771 biodiesel. Renewable Energy, 105, 637–646.

[29] Chiu, S. Y., Kao, C. Y., Chen, T. Y., Chang, Y. B., Kuo, C. M., Lin, C. S., 2015. Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource. Bioresource Technology, 184, 179-189.

[30] Cheirsilp, B., Thawechai, T., Prasertsan, P. (2017). Immobilized oleaginous microalgae for production of lipid and phytoremediation of secondary effluent from palm oil mill in fluidized bed photobioreactor. Bioresource Technology, 241, 787–794.

[31] Fazal, T., Mushtaq, A., Rehman, F., Ullah Khan, A., Rashid, N., Farooq, W., … Xu, J. (2018). Bioremediation of textile wastewater and successive biodiesel production using microalgae. Renewable and Sustainable Energy Reviews, 82, 3107–3126.

[32] Cecconet, D., Molognoni, D., Callegari, A., Capodaglio, A. G. (2017). Biological combination processes for efficient removal of pharmaceutically active compounds from wastewater: A review and future perspectives. Journal of Environmental Chemical Engineering, 5(4), 3590–3603.

[33] Torres, E. M., Hess, D., McNeil, B. T., Guy, T., Quinn, J. C. (2017). Impact of inorganic contaminants on microalgae productivity and bioremediation potential. Ecotoxicology and Environmental Safety, 139, 367–376.

[34] Leong, W. H., Lim, J.-W., Lam, M. K., Uemura, Y., Ho, C. D., Ho, Y. C. (2018). Co-cultivation of activated sludge and microalgae for the simultaneous enhancements of nitrogen-rich wastewater bioremediation and lipid production. Journal of the Taiwan Institute of Chemical Engineers, 87, 216–224.

[35] Kuo, C.M., Chen, T.Y., Lin, T.H., Kao, C.Y., Lai, J.T., Chang, J.S., Lin, C.S., 2015. Cultivation of *Chlorella* sp. GD using piggery wastewater for biomass and lipid production. Bioresource Technology, 194, 326-333.

[36] Feng, X., Walker, T.H., Bridges, W.C., Thornton, C., Gopalakrishnan, K., 2014. Biomass and lipid production of *Chlorella protothecoides* under heterotrophic cultivation on a mixed waste substrate of brewer fermentation and crude glycerol. Bioresource Technology, 166, 17-23.

[37] Levine, R. B., Costanza-Robinson, M. S., Spatafora, G. A. (2011). *Neochloris oleoabundans* grown on anaerobically digested dairy manure for concomitant nutrient removal and biodiesel feedstock production. Biomass and Bioenergy, 35, 40-49.

[38] Mu, J., Li, S., Chen, D., Xu, H., Han, F., Feng, B., Li, Y. (2015). Enhanced biomass and oil production from sugarcane bagasse hydrolysate (SBH) by heterotrophic oleaginous microalga *Chlorella protothecoides*. Bioresource Technology, 185, 99–105.

[39] Wang, Y., Ho, S.-H., Cheng, C.-L., Guo, W.-Q., Nagarajan, D., Ren, N.-Q., … Chang, J.-S. (2016). Perspectives on the feasibility of using microalgae for industrial wastewater treatment. Bioresource Technology, 222, 485–497.

[40] Chernova, N. I., Kiseleva, S. V. (2017). Microalgae biofuels: Induction of lipid synthesis for biodiesel production and biomass residues into hydrogen conversion. International Journal of Hydrogen Energy, 42(5), 2861–2867.

[41] Bich, N. N., Yaziz, M. I., Bakti, N. A. K. (1999). Combination of *Chlorella vulgaris* and *Eichhornia crassipes* for wastewater nitrogen removal. Water research, 33(10), 2357-2362.

[42] Ayyasamy, P. M., Banuregha, R., Vivekanandhan, G., Rajakumar, S., Yasodha, R., Lee, S., Lakshmanaperumalsamy, P. (2008). Bioremediation of sago industry effluent and its impact on seed germination (green gram and maize). World Journal of Microbiology and Biotechnology, 24(11), 2677-2684.

[43] Lim, J. W., Lim, P. E., Seng, C. E., Adnan, R. (2013). Alternative solid carbon source from dried attached-growth biomass for nitrogen removal enhancement in intermittently aerated moving bed sequencing batch reactor. Environmental Science and Pollution Research, 21(1), 485–494.

[44] Tolboom, S. N., Carrillo-Nieves, D., de Jesús Rostro-Alanis, M., de la Cruz Quiroz, R., Barceló, D., Iqbal, H. M. N., Parra-Saldivar, R. (2019). Algal-based removal strategies for hazardous contaminants from the environment – A review. Science of the Total Environment, 665, 358–366.

[45] Bai, X., Acharya, K. (2017). Algae-mediated removal of selected pharmaceutical and personal care products (PPCPs) from Lake Mead water. Science of The Total Environment, 581-582, 734–740.

[46] Xiong, J.-Q., Kurade, M. B., Jeon, B.-H. (2018). Can Microalgae Remove Pharmaceutical Contaminants from Water? Trends in Biotechnology, 36(1), 30–44.

[47] Cheng, D. L., Ngo, H. H., Guo, W. S., Chang, S. W., Nguyen, D. D., Kumar, S. M. (2018). Microalgae biomass from swine wastewater and its conversion to bioenergy. Bioresource technology, 275, 109-122.

[48] Wang, Y., Guo, W., Yen, H.-W., Ho, S.-H., Lo, Y.-C., Cheng, C.-L., … Chang, J.-S. (2015). Cultivation of *Chlorella vulgaris* JSC-6 with swine wastewater for simultaneous nutrient/COD removal and carbohydrate production. Bioresource Technology, 198, 619–625.

[49] Cheng, D. L., Ngo, H. H., Guo, W. S., Chang, S. W., Nguyen, D. D., Kumar, S. M., … Wei, D. (2018). Problematic effects of antibiotics on anaerobic treatment of swine wastewater. Bioresource Technology, 263, 642–653.

[50] Chinnasamy, S., Bhatnagar, A., Hunt, R. W., Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresource Technology, 101(9), 3097–3105.

[51] Kumar, M., Sun, Y., Rathour, R., Pandey, A., Thakur, I. S., Tsang, D. C. W. (2020). Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges. Science of The Total Environment, 137116.

[52] Cuevas-Castillo, G. A., Navarro-Pineda, F. S., Rodríguez, S. A. B., Rivero, J. C. S. (2020). Advances on the processing of microalgal biomass for energy-driven biorefineries. Renewable and Sustainable Energy Reviews, 125, 109606.

[53] Borowitzka, M. A. (2013). High-value products from microalgae—their development and commercialisation. Journal of applied phycology, 25(3), 743-756.

[54] Aravantinou, A. F., Theodorakopoulos, M. A., Manariotis, I. D. (2013). Selection of microalgae for wastewater treatment and potential lipids production. Bioresource Technology, 147, 130–134.

[55] Sydney, E. B., Sydney, A. C. N., de Carvalho, J. C., Soccol, C. R. (2019). Microalgal strain selection for biofuel production. Biofuels from Algae, 51–66.

[56] Borowitzka, M. (2018). Commercial-Scale Production of Microalgae for Bioproducts. Blue biotechnology: production and use of marine molecules, 1, 33-65.

[57] Griffiths, M. J., Harrison, S. T. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of applied phycology, 21(5), 493-507.

[58] Queiroz, M. I., Hornes, M. O., da Silva-Manetti, A. G., Jacob-Lopes, E. (2011). Single-cell oil production by cyanobacterium *Aphanothece microscopica Nägeli* cultivated heterotrophically in fish processing wastewater. Applied Energy, 88(10), 3438-3443.

[59] Osundeko, O., Ansolia, P., Gupta, S. K., Bag, P., Bajhaiya, A. K. (2019). Promises and Challenges of Growing Microalgae in Wastewater. Water Conservation, Recycling and Reuse: Issues and Challenges, 29–53.

[60] Neofotis, P., Huang, A., Chang, W., Joseph, F., Polle, J. (2016). Microalgae Strain Isolation, Screening, and Identification for Biofuels and High-Value Products. Microalgal Production for Biomass and High-Value Products, 63–89.

[61] Bharadwaj, S. V. V., Ram, S., Pancha, I., Mishra, S. (2020). Recent Trends in Strain Improvement for Production of Biofuels From Microalgae. Microalgae Cultivation for Biofuels Production, 211–225.

[62] Maroneze, M. M., Queiroz, M. I. (2018). Microalgal production systems with highlights of bioenergy production. In Energy from Microalgae (pp. 5-34). Springer, Cham.

[63] Suparmaniam, U., Lam, M. K., Uemura, Y., Lim, J. W., Lee, K. T., Shuit, S. H. (2019). Insights into the microalgae cultivation technology and harvesting process for biofuel production: A review. Renewable and Sustainable Energy Reviews, 115, 109361.

[64] Perez-Garcia, O., Escalante, F. M., de-Bashan, L. E., Bashan, Y. (2011). Heterotrophic cultures of microalgae: metabolism and potential products. Water research, 45(1), 11-36.

[65] Francisco, É. C., Franco, T. T., Wagner, R., Jacob-Lopes, E. (2014). Assessment of different carbohydrates as exogenous carbon source in cultivation of cyanobacteria. Bioprocess and biosystems engineering, 37(8), 1497-1505.

[66] Queiroz, M. I., Maroneze, M. M., Manetti, A. G. D. S., Vieira, J. G., Zepka, L. Q., Jacob-Lopes, E. (2018). Enhanced single-cell oil production by cold shock in cyanobacterial cultures. Ciência Rural, 48(11).

[67] Mohan, S. V., Rohit, M. V., Chiranjeevi, P., Chandra, R., Navaneeth, B. (2015). Heterotrophic microalgae cultivation to synergize biodiesel production with waste remediation: progress and perspectives. Bioresource technology, 184, 169-178.

[68] Wang, J., Yang, H., Wang, F. (2014). Mixotrophic cultivation of microalgae for biodiesel production: status and prospects. Applied biochemistry and biotechnology, 172(7), 3307-3329.

[69] Pandey, A., Srivastava, S., Kumar, S. (2019). Isolation, screening and comprehensive characterization of candidate microalgae for biofuel feedstock production and dairy effluent treatment: A sustainable approach. Bioresource technology, 293, 121998.

[70] Park, J. B. K., Craggs, R. J., Shilton, A. N. (2011). Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology, 102(1), 35–42.

[71] McBride, R. C., Lopez, S., Meenach, C., Burnett, M., Lee, P. A., Nohilly, F., Behnke, C. (2014). Contamination management in low cost open algae ponds for biofuels production. Industrial Biotechnology, 10(3), 221-227.

[72] Bagwell, C. E., Abernathy, A., Barnwell, R., Milliken, C. E., Noble, P. A., Dale, T., ... Moeller, P. D. (2016). Discovery of bioactive metabolites in biofuel microalgae that offer protection against predatory bacteria. Frontiers in microbiology, 7, 516.

[73] Clijsters, H., Van Assche, F. (1985). Inhibition of photosynthesis by heavy metals. Photosynthesis Research, 7(1), 31-40.

[74] Arita, C. E. Q., Peebles, C., Bradley, T. H. (2015). Scalability of combining microalgae-based biofuels with wastewater facilities: a review. Algal research, 9, 160-169.

[75] American Society for Testing and Materials (ASTM). ASTM 6751 – standard specification for biodiesel fuel (B100) blend stock for distillate fuels. West Conshohocken, PA, USA: ASTM; 2002.

[76] European Standard (EN). UNE-EN 14214 – automotive fuels – fatty acid methyl esters (FAME) for diesel engine - requirements and test methods. Pilsen, Czech Republic: European Standard; 2003.

[77] National ANP. Agency of petroleum, natural gas, and biofuels. Resolution ANP No. 45. 2014 dated 25.08 https://www.legisweb.com.br/legislacao/?id=274064. Accessed date: 8 April 2020.

[78] Singh, D., Sharma, D., Soni, S. L., Sharma, S., Kumari, D. (2019). Chemical compositions, properties, and standards for different generation biodiesels: A review. Fuel, 253, 60-71.

[79] Maroneze, M. M., Barin, J. S., Menezes, C. R. D., Queiroz, M. I., Zepka, L. Q., Jacob-Lopes, E. (2014). Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors. Scientia Agricola, 71(6), 521-524.

[80] Lobo, I. P., Ferreira, S. L. C., da Cruz, R. S. (2009). Biodiesel: Quality parameters and analytical methods. Química nova, 32(6), 1596-1608.

[81] Khan, M. I., Shin, J. H., Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial cell factories, 17(1), 36.

[82] Jacob-Lopes, E., Franco, T. T. (2013). From oil refinery to microalgal biorefinery. Journal of CO_2 utilization, 2, 1-7.

[83] Dias, R. R., Vieira, K. R., Pinheiro, P. N., Zepka, L. Q., Jacob-Lopes, E. (2019). Biodiesel from microalgae. In: A closer look at biodiesel production, 1-319.

[84] Amaro, H. M., Macedo, Â. C., Malcata, F. X. (2012). Microalgae: An alternative as sustainable source of biofuels?. Energy, 44(1), 158–166.

[85] Shah, S. H., Raja, I. A., Rizwan, M., Rashid, N., Mahmood, Q., Shah, F. A., Pervez, A. (2018). Potential of microalgal biodiesel production and its sustainability perspectives in Pakistan. Renewable and Sustainable Energy Reviews, 81, 76–92.

[86] Zhou, X., Yuan, S., Chen, R., Ochieng, R. M. (2015). Sustainable production of energy from microalgae: review of culturing systems, economics, and modelling. Journal of Renewable and Sustainable Energy, 7(1), 012701.

[87] Beal, C. M., Gerber, L. N., Sills, D. L., Huntley, M. E., Machesky, S. C., Walsh, M. J., … Greene, C. H. (2015). Algal biofuel production for fuels and feed in a 100-ha facility: A comprehensive techno-economic analysis and life cycle assessment. Algal Research, 10, 266–279.

[88] Sun, J., Xiong, X., Wang, M., Du, H., Li, J., Zhou, D., Zuo, J. (2019). Microalgae biodiesel production in China: A preliminary economic analysis. Renewable and Sustainable Energy Reviews, 104, 296-306.

[89] Xin, C., Addy, M. M., Zhao, J., Cheng, Y., Cheng, S., Mu, D., … Ruan, R. (2016). Comprehensive techno-economic analysis of wastewater-based algal biofuel production: A case study. Bioresource Technology, 211, 584–593.

[90] Milano, J., Ong, H. C., Masjuki, H. H., Chong, W. T., Lam, M. K., Loh, P. K., Vellayan, V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. Renewable and Sustainable Energy Reviews, 58, 180-197.

[91] Delrue, F., Setier, P. A., Sahut, C., Cournac, L., Roubaud, A., Peltier, G., Froment, A. K. (2012). An economic, sustainability, and energetic model of biodiesel production from microalgae. Bioresource technology, 111, 191-200.

[92] Chen, J., Li, J., Dong, W., Zhang, X., Tyagi, R. D., Drogui, P., Surampalli, R. Y. (2018). The potential of microalgae in biodiesel production. Renewable and Sustainable Energy Reviews, 90, 336-346.

[93] IEA. International Energy Agency. Oil market report (2020). https://www.iea.org/reports/oil-market-report-may-2020, Accessed 17 May 2020.

[94] Subhadra, B. G., Edwards, M. (2011). Coproduct market analysis and water footprint of simulated commercial algal biorefineries. Applied Energy, 88(10), 3515-3523.

[95] Onukwuli, D. O., Emembolu, L. N., Ude, C. N., Aliozo, S. O., Menkiti, M. C. (2017). Optimization of biodiesel production from refined cotton seed oil and its characterization. Egyptian Journal of Petroleum, 26(1), 103-110.

[96] Ventura, J. R. S., Yang, B., Lee, Y. W., Lee, K., Jahng, D. (2013). Life cycle analyses of CO_2, energy, and cost for four different routes of microalgal bioenergy conversion. Bioresource technology, 137, 302-310.

[97] Jacob-Lopes, E., Maroneze, M. M., Deprá, M. C., Sartori, R. B., Dias, R. R., Zepka, L. Q. (2019). Bioactive food compounds from microalgae: an innovative framework on industrial biorefineries. Current Opinion in Food Science, 25, 1-7.

[98] El Shimi, H. I., Moustafa, S. S. (2018). Biodiesel production from microalgae grown on domestic wastewater: Feasibility and Egyptian case study. Renewable and Sustainable Energy Reviews, 82, 4238-4244.

Table 1 - Types of residues and characteristics favorable to microalgal lipid production.

Wastes	**Composition Characteristics of Waste**	**Reference**
Secondary effluents from palm oil mill	Nitrogen and inorganic phosphorus	[30]
Textile wastewater	Organic and inorganic nutrients, nitrate and phosphate anions, carbon	[2,31]
Pharmaceutically wastewater	Pharmaceutically active compounds (PhACs) including a wide range of compounds used to prevent/treat human and animal diseases, Pharmaceuticals and personal care products (PPCPs)	[32]
Sewage sludge	Nitrifiers, inorganic contaminants	[33,34]
Swine wastewater	Suspended solids, organic materials, heavy metals, antibiotics and hormones	[35]
Broth mixture of beer and fermentation residues crude glycerol	High level of nitrogen, glycerol, carbon	[36]
Anaerobically digested milk manure	Organic and inorganic nutrients, high turbidity, competitive microorganisms, phosphorus and NH_4^+	[37]
Cane bagasse hydrolyzate	Xylose, arabinose and glucose	[38]

Rubber wastewater	Washing water, protein whey, non-coagulated latex, lipids, carbohydrates, salts, chemical and biochemical oxygen demand, ammonia, nitrate, phosphorus and total solids.	[8]

Adapted from Ref. [19].

Table 2 - Fatty acid profile in different biodiesel feedstocks.

	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2n6	C18:3n6	C20:0	C20:1	C22:1
					1st generation					
Almond Kernel	-	-	6.5	1.4	70.7	20	0,9	-	-	-
Soybean	-	-	6-10	2-5	20-30	50-60	5-11	-	-	-
Coconut	45-53	16-21	7-10	2-4	5-10	1-2.5	-	-	-	-
Mustard	-	-	-	1-2	8-23	10-24	8-18	-	5-13	20-50
Olive	-	-	9-10	2-3	72-85	10-12	0-1	-	-	-
					2nd generation					
Cottonseed	-	-	22.9-28.3	0.8-0.9	13.27-18.3	-	0.2	-	-	-
Jatropla	-	14.1-15.3	0-13	-	34.3-45.8	14.1-15.3	0-0.3	-	-	-
Karanja	-	-	3.7-7.9	2.4-8.6	44.5-71.3	10.8-18.3	-	-	-	-
Linseed	-	-	4-2	2-4	24-40	35-40	25-60	-	-	-
Neem	-	-	13.6-16.2	-	49-62	-	-	-	-	-
					3rd generation					
Chicken fat	-	3.1	19-82	3.1	37.6	-	-	-	-	-
Arthrospira platensis	-	7.50	25.0	7.7	34.7	12.5	8.2	-	-	-
Scenedesmus obliquus	-	-	34.0	2.6	5.7	1.7	0.4	-	-	-
Chlorella vulgaris	0.9	6.1	22.6	21.4	6.9	6.6	14.3	2.3	6.0	-

Adapted from Refs. [13,14,78].

Table 3 - Properties of the biodiesel reasoned on EU (EN), USA (ASTM), and Brazilian (ANP) standards and biodiesel properties produced by microalgae.

	ASTM D6751	**EN 14214**	**ANP 45**	**Microalgae Sludge**
Ester content	-	≤96.5%	≤96,5%	99%
Density	-	860–900 kg/m^3	850 a 900 kg/m^3	-
Viscosity	1.9–6 (mm^2 /s)	3.5–5.0 (mm^2 /s)	3.0–6.0 (mm^2 /s)	-
Flash point	≥130°C	≥101 °C	≥100 °C	-
Sulfur content	≤50 (mg/kg)	≤10 (mg/kg)	≤10 (mg/kg)	-
Carbon residue	≤0.05 (m/m %)	≤0.3 (m/m %)	-	-
Cetane number	≥47	≥51	-	55
Water content	≤0.05 (v/v %)	≤500 (mg/kg)	≤200 (mg/kg	-
Copper strip corrosion as degree of corrosion	3h	-	-	-
Oxidation stability	≥3 h	≥4 h	≥6 h	-
Acid value	≤0.50 (mg KOH/g)	≤0.50 (mg KOH/g)	≤0.50 (mg KOH/g)	-
Iodine value	-	130 ($gI_2100\ g^{-1}$)	-	73.5 ($gI_2100\ g^{-1}$)
Methanol content	-	≤0.02 (m/m %)	≤0.02 (m/m %)	-
Monocylglycerols	-	– ≤0.80 (mole %)	≤0.70 (mole %)	-
Diacylglycerols	-	≤0.20 (mole %)	≤0.20 (mole %)	-
Triacylglycerols	-	≤0.20 (mole %)	≤0.20 (mole %)	-

Degree of unsaturation	-	-	-	75%
Free glycerin	≤0.20 (m/m %)	≤0.02 (mole %)	≤0.02 (mole %)	-
Total glycerin	≤0.25 (m/m %)	≤0.25 (m/m %)	≤0.25 (m/m %)	-
Pour point	−15–16°C	-	-	-
Phosphorus	≤0.001 (m/m %)	≤4 (mg/kg)	≤10 (mg/kg)	-
Cloud point	−3–12°C	-	-	-

Adapted from Refs. [13,79].

Figure 1 - General process flow diagram of microalgae biodiesel production.

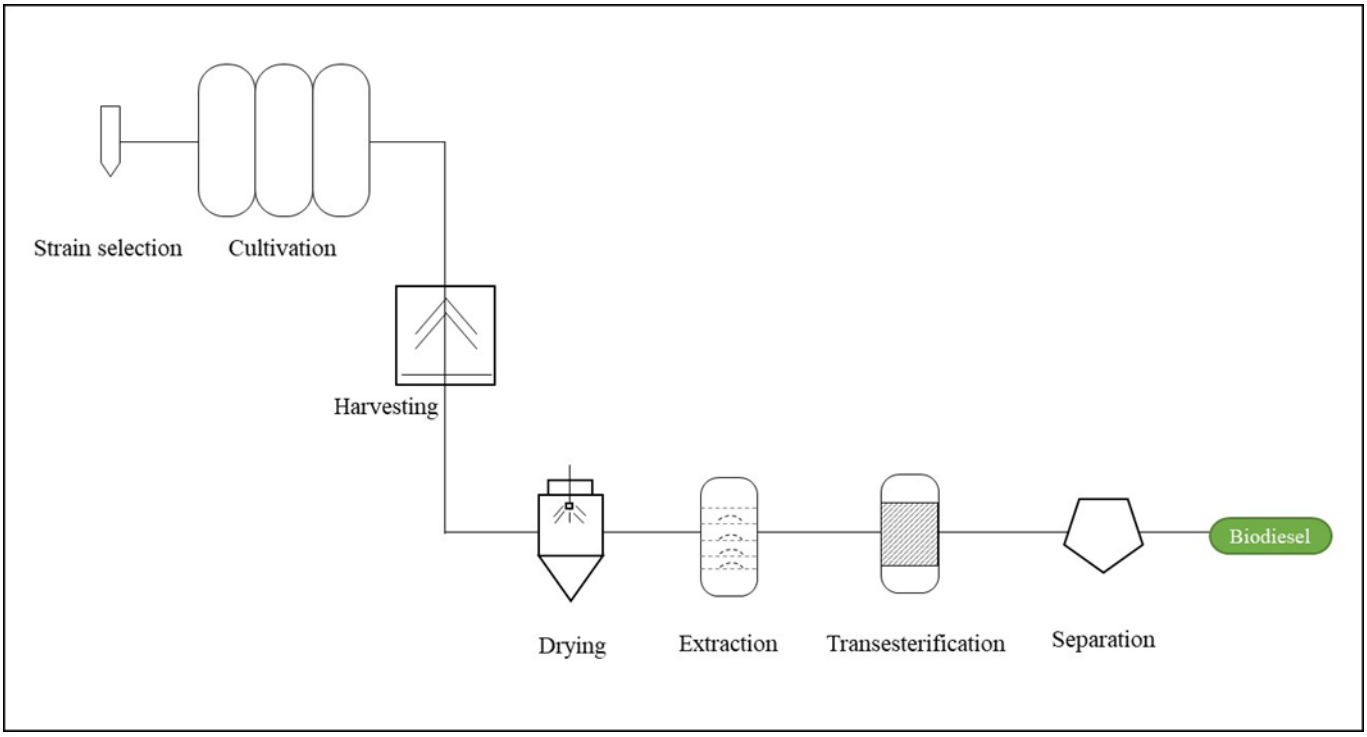
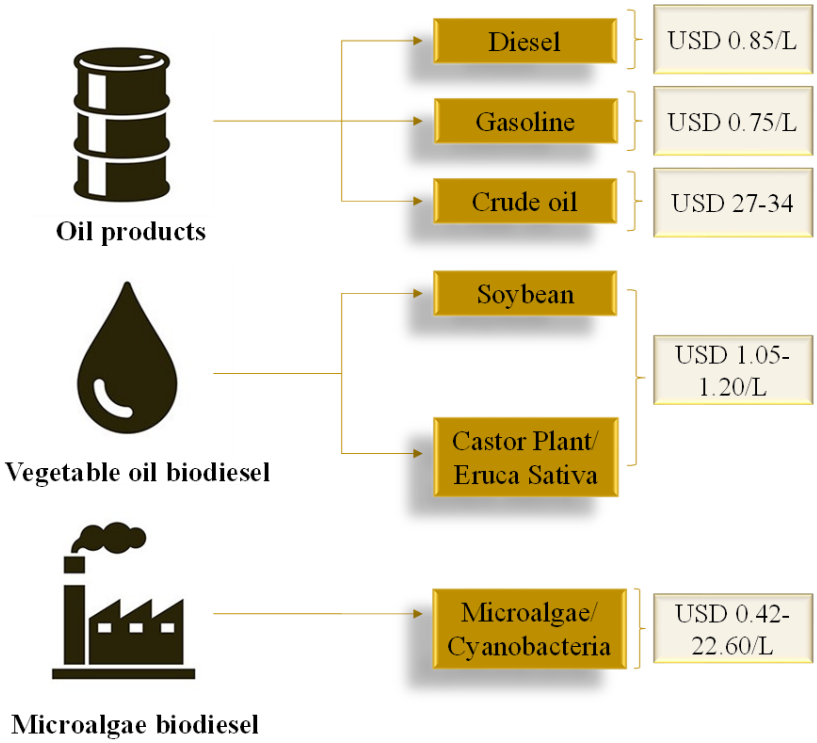


Figure 2 - Price of classic products manufactured through refinements and traditional biorefineries.



Adapted from Refs. [1,13,92-94].

5 CONCLUSÃO GERAL

A microalga *Chlorella vulgaris* CPCC90, apresentou diferenças em relação ao seu perfil qualitativo e quantitativo de carotenoides nas diferentes fases de crescimento. Um total de 23 carotenoides foi identificado e a maior produção heterotrófica total de carotenoides foi em 4h com 1612,12µg/g (peso seco). O carotenoide majoritário em todo o experimento foi all-*trans*-luteína. A partir deste trabalho, é possível recomendar que espécies do gênero *Chlorella* sejam candidatas adequadas para a produção heterotrófica de luteína e demonstrar que os carotenoides também são sintetizados no escuro, tornando a cultura heterotrófica uma opção interessante para a produção comercial desses compostos.

Microalgae Application in Chemicals, Enzymes, and Bioactive Molecules

14

Paola Lasta, Patricia Arrojo da Silva, Patricia Acosta Caetano, Pricila Nass Pinheiro, Leila Queiroz Zepka, and Eduardo Jacob-Lopes

Abstract

Microalgae feature the ability to develop in different ecosystems, consequently because they are photosynthetic microorganisms with a simple structure. Recently, the interest production of microalgae-based products has increased, due to the integrity of these natural microorganisms in the production of fatty acids, lipids, carbohydrates, pigments, proteins, vitamins, antioxidants, enzymes, and bioactive molecules. It is crucial to study cultivation systems, species, and environmental factors, as they may have strong mastery over the cultivation of microalgae. Microalgae require cheap substrates, such as sunlight, temperature, and carbon dioxide, being used as affordable and effective biocatalysts to obtain products with high added value and commercial applicability (nutraceuticals, pharmaceuticals, biofuels, cosmetics, and functional foods, among others). Therefore, this chapter reports on the mechanisms of formation, production, and application of these components from microalgae (chemicals, enzymes, and bioactive molecules), in addition to providing a description of microalgae-based products, improving the application of microalgal biomass in several segments.

Keywords

Microalgae · Microalgae-based products · Chemicals · Enzymes · Bioactive molecules · Industrial applications

P. Lasta · P. A. da Silva · P. A. Caetano · P. N. Pinheiro · L. Q. Zepka · E. Jacob-Lopes (✉)
Department of Food Science and Technology, Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil

Inamuddin et al. (eds.), *Application of Microbes in Environmental and Microbial Biotechnology*, Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-16-2225-0_14

14.1 Introduction

Microalgae are considered photosynthetic microorganisms being able to grow in marine or freshwater systems with applications in industrial units (Pignolet et al. 2013). The classification of microalgae includes prokaryotic and eukaryotic microalgae (Borowitzka 2013). According to Gimpel et al. (2015), there are 40,000–70,000 species of microalgae referring to 9 classes, with species not yet discovered or classified.

As photosynthetic microorganisms, microalgae are considered valuable sources for many applications, through biomass, production of various compounds, and environmental applications. Commercial exploitation by these microorganisms has increased due to the need for reliable, efficient, and economical processes (Fernandes et al. 2015).

Microalgae are used to obtain compounds with high added value, requiring only sunlight, temperature, and carbon dioxide (CO_2), for their superior growth (Vilchez et al. 1997). In addition, numerous strains of microalgae produce compounds such as lipids being possibly converted into biodiesel, and microalgae biomass is characterized by having valuable compounds, such as carbohydrates, fatty acids, pigments, proteins, vitamins, and antioxidants, favoring the transformation of these compounds into refined products for various segments (Nur and Buma 2019; Koller et al. 2014).

However, some factors influence the behavior of microalgae, such as high cost of installation and operation, difficulty in controlling culture conditions, contaminating microorganisms, unstable light supply, and local climate (Yen et al. 2013). Therefore, the classes of microalgae and their adaptation changes in climatic factors, in particular light and temperature, must be studied to obtain a successful, economical, and sustainable process (Bhalamurugan et al. 2018).

The industry is focused on expanding products for human nutrition, animal feed, aquaculture food, cosmetic products, pigments, biofertilizers, medicines, and biofuels. Notably, microalgae are producers of many important biochemicals that have not yet been discovered (Rizwan et al. 2018).

Therefore, this chapter addresses an overview of the mechanisms of formation, production, and applications of these components based on microalgae (chemicals, enzymes, and bioactive molecules). In addition, it provides a description of the microalgae-based products generated and their application in various commercial segments.

14.2 Microalgae-Based Products

14.2.1 Chemical Products

Several species of microalgae are considered promising candidates for obtaining useful materials, such as biofuels and chemicals; from this perspective, there is a great demand for more natural and sustainable products (Maeda et al. 2018).

Microalgae are microorganisms capable of accumulating macromolecules, such as proteins, lipids, and carbohydrates, through the capture of solar energy, CO_2, and nutrients. Besides, they are widely used in contemporary nutraceutical foods, through their ability to synthesize aggregate products such as pigments (carotenoids), essential and non-essential amino acids, sugar, enzymes, fatty acids, essential vitamins, and minerals for human consumption (Matos 2017).

These chemical compounds of high added value can be extracted from different microalgae species, being used as bulk commodities in several industrial sectors. In order to obtain chemical products and bioactive compounds, it is essential to cultivate suitable species, together with cultivation systems and ideal conditions, to acquire the desired final product (Mata et al. 2010).

As shown in Fig. 14.1, the productivity of microalgae biomass can be directed to various industrial segments as a source of healthy food, a source of protein for fish farming, a source of animal feed (cattle, swine and poultry), production of cosmetics, medicines and biodiesel (Koller et al. 2012).

The processing of microalgae occurs in three stages: cultivation, harvesting, and extraction. However, the cultivation mode and the choice of species are of paramount importance to define the desired final product (Rizwan et al. 2018). Today, outdoor cultivation is the most economical and viable system in terms of energy and operating costs (Maeda et al. 2018).

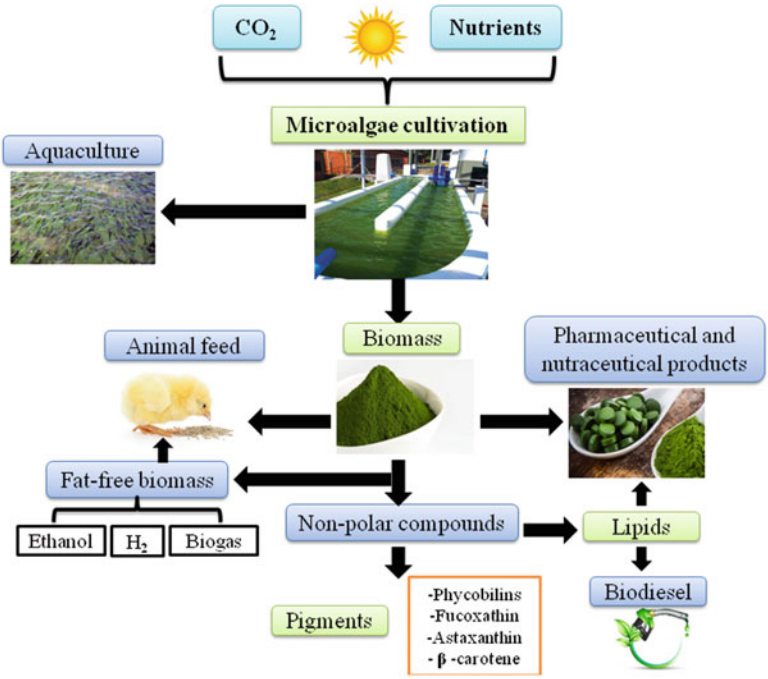


Fig. 14.1 Cultivation of microalgae to generate products with high added value with different industrial segments. (Adapted from Bellou et al. (2014))

The environmental conditions are determining factors for the development of microalgae cultures. In systems exposed to the outdoors, it is imperative to control the parameters, mainly for the generation of biomass (Eriksen 2008). Climatic factors such as carbon dioxide, sunlight, water, temperature, and nutrients are indispensable for the development of microalgae (Chisti 2007). These factors present daily and seasonal variations according to the climatic and geographic location; however, many species behave differently in the face of limiting factors (Bellou et al. 2014).

However, in systems exposed to the outdoors, it is not possible to control the temperature and light intensity, which vary during the day and throughout the year. Therefore, integration technologies and systems engineering are presented, which can be used to optimize the microalgae growth control system and, thus, thrive under ideal conditions (Zhu and Hiltunen 2016).

Notably, when choosing the biomass harvesting method, it is necessary to analyze the profile of the microalgae and their cultivation conditions. So far, the harvesting modes found are flocculation, centrifugation, filtration, sedimentation, and flotation. The capacity of the methods depends on the microalgae strains, including the size, morphology, and composition of the medium used (Japar et al. 2017). After harvesting, the biomass is subjected to the extraction process, obtaining valuable products to produce compounds with high added value (Olguín 2012). In this sense, Fig. 14.2 illustrates several methods of extraction for different chemicals obtained by microalgae.

More specifically, microalgae lipids are divided into storage lipids (triglycerides) and structural lipids (sterols and phospholipids) (Levasseur et al. 2020). However, the increase in lipid production for the generation of biofuels contributes to the sustainability and competitiveness of the microalgae market (Bekirogullari et al. 2017). In this perspective, biodiesel has many benefits, being able to reduce emissions of carbon monoxide, carbon dioxide, and sulfur into the atmosphere. Notably, biodiesel is biodegradable, non-toxic, and own similarities to conventional diesel, such as energy content and chemical and physical properties (Pragya et al. 2013).

Microalgae are fatty acid producers with a high degree of unsaturation and unusual chain lengths, besides to not being found in natural quantities or elsewhere (Hess et al. 2018); examples of fatty acids obtained from microalgae are arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and linolenic acid, being useful to treat diverse disease and as a food source. Several species of microalgae feature the capacity to produce significant amounts of oils and fats, such as omega-3 and omega-6. Currently, DHA is the only microalgae PUFA produced on a commercial scale (Rizwan et al. 2018).

Carbohydrates are divided into sugars (monosaccharides) and polymers (disaccharides, oligosaccharides, and polysaccharides) (Markou et al. 2012). Some strains of microalgae have a high content of carbohydrates (starch and cellulose), being excellent substrates for the generation of bioethanol; the use of carbohydrate to obtain bioethanol becomes advantageous because microalgae proliferate and fix CO_2 at a higher rate compared to other terrestrial plants (Ho et al. 2013).

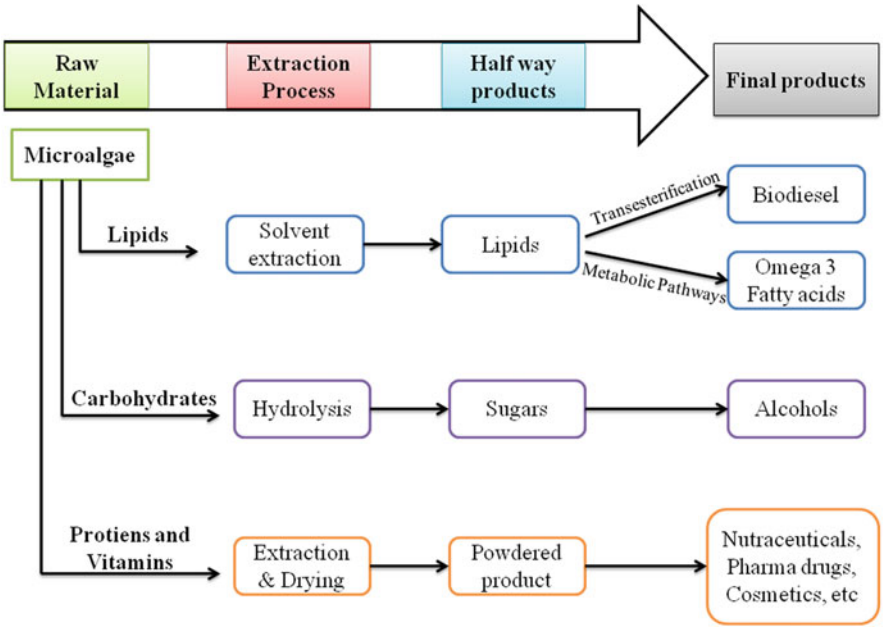


Fig. 14.2 Different chemical products based on microalgae by various extraction processes. (Adapted from Enamala et al. (2018))

Microalgal proteins are similar to food proteins, consequently, due to the excellent profile and compatibility of amino acids, they are used in the pharmaceutical industry to treat some diseases. On the other hand, proteins are defined by their low index of stability and denaturation under acidic and alkaline conditions, making extraction and separation difficult (Markou and Nerantzis 2013; Chew et al. 2017).

Microalgae feature a great capacity to produce several essential vitamins, for example, A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid, and pantothenic acid; therefore, the index of microalgal vitamins is of high interest for application in food (Graziani et al. 2013). The number of vitamins is more concentrated in microalgae than in conventional foods (Fabregas and Herrero 1990).

14.2.2 Bioactive Molecules

Bioactive molecules are biologically active substances presenting desirable features in human health. Currently, there is growing interest in the generation of bioactive molecules from natural products through the use of microalgae biomass, driven by a growing body of research demonstrating the beneficial approaches of bioactive molecules to health (Ejike et al. 2017).

The market for bioactive food compounds by microalgae is an opportunity in the segment of bioactive molecules, dominated by synthetic substances and sources of animals and plants (Jacob-Lopes et al. 2019). This composition of biomolecules can be designated as a bioproduct, rich in macro- and micronutrients. Thus, studies show microalgae are an innovation biotechnological applications in industrial sectors related to biofuel, chemical, pharmaceutical, cosmetics, and food (Rodrigues et al. 2015; de Morais et al. 2020). Table 14.1 demonstrates the main bioactive molecules extracted from microalgae (Table 14.1).

These photosynthetic microorganisms can accumulate significant natural bioactive compounds. Among these molecules, natural pigments are the most exciting components produced. Its main classes of phytonutrients are carotenoids, chlorophyll, and phycobiliproteins (Rodrigues et al. 2015). Derivatives of carotenoids can be isolated, and the main include is neoxanthin, violaxanthin, lutein, zeaxanthin, canthaxanthin, mixoxanthophyll, echinenone, (all-*E*)-α-carotene, (all-*E*)-β-carotene, and also its isomeric structures (*Z*). Derived pigments that are produced only by microalgae are echinenone, mixoxanthophyll, and canthaxanthin with antioxidant potential (Nascimento et al. 2020a).

Table 14.1 The main bioactive compounds from microalgae

Bioactive compounds	Microalgae
All-*trans*-β-carotene, all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-canthaxanthin, all-*trans*-myxoxanthophyll, all-*trans*-echinenone, chlorophyll *a,* chlorophyll *b, and* phycobiliproteins	*Phormidium autumnale*
Polysaccharides, phycocyanin, C-phycocyanin, allophycocyanin, phenolic acids, tocopherols (vitamin E), neophytadiene, phytol, PUFAs (*n*-3) fatty acids, oleic acid, linolenic acid, palmitoleic acid, diacylglycerols, terpenoids, alkaloids, and flavonoids	*Spirulina* sp., *S. platensis, S. fusiformis, S. maxima*
All-*trans*-β-carotene, all-*trans*-zeaxanthin, all-*trans*-lutein, *cis*-beta carotene, oleic acid, linolenic acid, palmitic acid, diacylglycerols, and sterols	*Dunaliella salina*
All-*trans*-astaxanthin, all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-canthaxanthin, all-*trans*-β-carotene, and oleic acid	*Haematococcus pluvialis*
Carotenoids, sulfated polysaccharides, sterols, PUFAs (n-3) fatty acids, all-*trans*-canthaxanthin, all-*trans*-astaxanthin, peptide, oleic acid, eicosapentaenoic acid (EPA), all-*trans*-violaxanthin, all-*trans*-lutein, phenolic, terpenoids, alkaloids, phytol, and phenol	*Chlorella* sp., *C. vulgaris*, *C. minutissima*, *C. ellipsoidea*, *C. protothecoides*
Protein (bioactive peptides), all-*trans*-β-carotene, and all-*trans*-lutein	*Scenedesmus obliquus*
Borophycin, cryptophycin, phycocyanin, phenolic, terpenoids, alkaloids, and phycobilins	*Nostoc* sp., *N. muscorum*, *N. humifusum*, *N. linckia*, *N. spongiaeforme*

Adapted from de Morais et al. (2020) and Nascimento et al. (2020b)

The β-carotene is well known to have the highest provitamin A activity (Raposo et al. 2013a). Natural pigments have beneficial health-related properties. Their antioxidant activity balances the harmful effects of free radicals that have been associated with reduced risk of developing several degenerative diseases (da Silva Vaz et al. 2016).

The studies promising pharmacological action bioactivity of chlorophylls compounds is during the photodynamic therapy. There is also, evidences supporting that the role of chlorophyll derivatives can rebalance the gut microbiota (Zepka et al. 2019).

Phycobiliproteins are pigments hydrophilic protein complexes found in microalgae with highly sensitive fluorescent properties that are comprised of C-phycocyanin, phycoerythrin, and allophycocyanin and thus can be used as a detector for specific pharmacological analysis (Levasseur et al., 2020).

Microalgal proteins are sources of alternative nutrition and easy digestibility, acting as antioxidants and antimicrobials, thereby alternative to a healthy diet due to their bioactive peptide, amino acid, fatty acid, and phycobiliprotein content (Zepka et al. 2010). Therefore, when are inserted into a diet, compounds become bio-based decreasing body weight and preventing diet-induced obesity (Patias et al. 2018). The *Spirulina* species, helps in the treatment of many diseases as a result of its exceptional antioxidant, antibacterial, anti-tumor, immunoprotection, and anti-inflammatory properties and also reduces appetite and improves food absorption (Moradi et al. 2019).

The microalgae strain can have a wide range of sterols, from cholesterol to β-sitosterol. These compounds become important due to their antioxidant, anticarcinogenic and anti-inflammatory activity (Fagundes et al. 2020).

Microalgal polysaccharides produce original biopolymers with unique structures and composition to obtain sulfate esters, which are referred to as sulfated polysaccharides (carrageenan, ulvan, and fucoidan), and exhibit various bioactivities, such as antiviral, antioxidant, and anti-inflammatory activities. The production of macromolecules represents high-value products with applications in cosmetics, emulsifiers, food, fabrics, medicines, and stabilizers. Studies are being proposed to use microalgal polysaccharides as a promising prebiotic fiber source (Tang et al. 2020).

But also, according to Lafarga et al. (2020), the microalgae contains a wide spectrum of prophylactic and pharmaceutical phytonutrients including excellent sources of vitamins and minerals. Additionally, there is a lot of attractive biochemical profile that needs better exploited, being the enzymes. Among microorganisms, microalgae become a promising source for future research (Rocha et al. 2018).

14.3 Microalgae Enzymes

The potential application of microalgal biomass extends beyond the bioproducts established to date. There is still great untapped timeliness for utilizing this resource. Indeed, the synthesis of enzymes by microalgae has been recently proposed as a

potential niche for the generation of amylases, proteases, lipases, peroxidases, laccases, phytases, and galactosidases (Brasil et al. 2017; Ellatif et al. 2020; Spier et al. 2020).

Amylases belong to a series of glycohydrolase enzymes acting the carbohydrate hydrolysis reaction (Azzopardi et al. 2016; Mohanan and Satyanarayana 2019). Amylases were the first enzymes employed for industrial processes, with large-scale production. Its global market value was estimated at US $ 1.6 billion in 2020, with the largest commercial share of 25%–30% (Mehta and Satyanarayana 2016; Cripwell et al. 2020). Thus, amylases are applied in numerous segments, including the food industry (e.g., in the cheese ripening, baking, chocolate, infant cereal, and brewing and as flavoring), the pharmaceutical industry (high-fructose syrups), the textile and paper industries, and the manufacture of detergents and bioethanol (Brasil et al. 2017; Cripwell et al. 2020; Spier et al. 2020).

Among the enzymes described in microalgae, amylases are the least reported; this is due to their autotrophic metabolism (Patil et al. 2001). However, the species *Chlorella sorokiniana*, *Chlamydomonas reinhardtii*, *Dallina parva*, *Dunaliella tertiolecta*, *Dunaliella marina*, *Klebsormidium* sp., *Oedogonium* sp., *Rhizoclonium* sp., *Rhizoclonium hieroglyphicum*, *Scenedesmus obliquus*, and *Spirogyra* sp. demonstrated amylase activity (Kombrink and Weober 1980; Levi and Gibbs 1984; Patil and Mahajan 2016; Manoj et al. 2018).

Proteases are enzymes that catalyze hydrolytic reactions, resulting in the cleavage of protein molecules into peptides and amino acids and representing the second largest group in market volume. Proteases are extensively exploited in the cleaning, food, and textile manufacturer (Aguilar and Sato 2018; Sharma et al. 2017).

Microalgae studies have shown that protease activity may be related to environmental factors, such as luminosity or nutrient restriction, nitrogen source, and cell apoptosis (Brasil et al. 2017; Spier et al. 2020). Niven (1995) determined the influence of different nutrient sources on the protease activity in *Anabaena variabilis*. In turn, Lockau et al. (1988) and Strohmeier et al. (1994) explored the same microorganisms and their dependence on calcium in the production of protease. Moreover, protease activity has also been observed in *Chlorella vulgaris* and *Arthrospira platensis* (Nanni et al. 2001; Yada et al. 2005; Silva et al. 2017).

Lipases are important biocatalysts due to their capability to hydrolyze triglyceride into fatty acids and glycerol. Accordingly, lipases have attracted commercial attention, falling only behind amylases and proteases in terms of global enzyme sales. The technical features of these enzymes have enabled its introduction in numerous applications in the food, animal feed, pharmaceutical, detergent, paper, cellulose, and bioremediation industries (Brasil et al. 2017; Almeida et al. 2020; Spier et al. 2020).

The lipases investigated in *Botryococcus sudeticus* and *Isochrysis galbana* have promising characteristics for industrial applications, such as substrate specificities, pH endurance (pH 5–11), and temperature resistance (40–70 °C). Furthermore, microalgae species *Arthrospira platensis* and *Nannochloropsis oceanica* also demonstrated the activity of this enzyme (Demir and Teukel 2010; Godet et al.

2012; Savvidou et al. 2016; Yong et al. 2016; Brasil et al. 2017; Hubert et al. 2017; Spier et al. 2020).

Peroxidases are antioxidant enzymes that catalyze the redox reaction for various substrates. Therefore, peroxidases are deliberated a valuable catalyst for several medicinal, industrial, and bioremediation applications (e.g., decolorization of synthetic textile effluents) (Medina et al. 2016). The peroxidases activity was observed in some microalgae strains, as *Coelastrella* sp., *Dunaliella tertiolecta*, *Galdieria sulphuraria*, *Euglena gracilis*, *Phaeodactylum tricornutum*, *Rhizoclonium* sp., *Oedogonium* sp., and *Porphyridium purpureum* (Overbaugh and Fall 1982; Overbaugh and Fall 1985; Murphy et al. 2000; Oesterhelt et al. 2008; Baldev et al. 2013).

The laccase enzyme, act on the oxidation of complex substrates (e.g., phenols and aliphatic or aromatic amines) with the concurrent reduction of a molecule of oxygen and releasing water molecules (Li et al. 2020; Spier et al. 2020). Laccases are widely involved in bioremediation processes of brewing effluents, paper, textile, and pulp (Brasil et al. 2017). Thus, the species *T. aeria* and *C. moewusii* are investigated for the biodegradation of phenolic pollutants in industrial wastewaters (Otto et al. 2015). Moreover, these enzymes have also been described in *Phormidium valderianum*, *Arthrospira platensis*, and *Oscillatoria boryana* (Otto et al. 2010; Afreen et al. 2017; Ellatif et al. 2020).

Phytase enzymes catalyze the hydrolysis of phytate through a series of myo-inositol phosphate intermediate compounds and inorganic phosphate. The phytase own several applications in the industries, mostly in the food manufacturer, where they are used in the elaboration of animal feed, aiming at cost reduction, minimizing the environmental impact, increasing the phosphorus bioavailability, and decreasing the anti-nutrition effect of phytate in monogastric animals (Handa et al. 2020; Sharma et al. 2020).

Due to the commercial appeal of this enzyme, the transgenic microalgae *Chlamydomonas reinhardtii* were studied for the exploration of phytase at a suitable pH and gastrointestinal temperature that can be applied as food supplements. However, other investigated species, such as *C. thermalis Geitler*, *S. bigranulatus Skuja*, and *S. lividus*, also demonstrated phytase activity (Klanbut et al. 2002; Erpel et al. 2016).

Galactosidases are a family of glycoside hydrolase enzymes that further the hydrolysis the glycosidic bonds (Naumoff 2011; Vidya et al. 2020). The enzymes α-galactosidase and β-galactosidase are important glycoside hydrolases with employment in the food, feed, and pharmaceutical industries (Husain 2010; Zhao et al. 2018). The enzyme α-galactosidase aims to hydrolyze the α-galactosyl (α 1–6 linkages) terminal moieties of glycolipids and glycoproteins, whereas, β-galactosidase clive the D-galactosyl (β 1–4 linkages) residues from oligosaccharides or polymers (Spier et al. 2020; Vidya et al. 2020).

In microalgae, α-galactosidase activity was observed in *Poterioochromonas malhamensis* as a metabolic result of external osmotic pressure (Dey and Kauss 1981). On the other hand, the microalgae *C. minutissima*, *D. tertiolecta*, *N. oculata*, *S. obliquus*, and *T. obliquus* demonstrated the formation of β-galactosidase (Davies

et al. 1994; Girard et al. 2014; Bentahar et al. 2018; Suwal et al. 2019; Zanette et al. 2019).

Therefore, microalgae can metabolize a wide pool of enzymes, proving how these species are versatile. However, the microalgae are still little explored in comparison to other microorganisms, but numerous enzymes are being investigated and can be applied in various sectors of the industry (Brasil et al. 2017; Spier et al. 2020).

14.4 Industrial Applications of Microalgae

Due to innumerable scientific studies, microalgae have shown great potential as an alternative source for several operations through the bio-refining procedure. Today, microalgae are applied in various industrial sectors, due to their high survival skills in aggressive environments of temperature, pH, light intensity, salinity, and accelerated growth rate (Bhattacharya and Goswami 2020; Tang et al. 2020; Geada et al. 2017). Microalgae are promising for the generation of biodiesel and other products, including feed, nutraceuticals, and food (Giordano and Wang 2018; Rahman 2020).

The world trade in algae biomass is estimated at about US $ 3.8 to 5.4 billion, and approximately 7000 tons of dry algae biomass are manufactured globally (Brasil et al. 2017). In addition, the data indicate that the algae trade is becoming increasingly popular and has the potential to be applied to various branches of the industrial sector afterward (Tang et al. 2020). Today, the United States, Asia, and Oceania control the microalgae generation trade. Despite this, research indicates that Europe is likely to become a significant powerhouse in the field of microalgae bioproducts in the future (Rahman 2020).

Currently, the introduction of synthetic compounds in food, cosmetics, and pharmaceutical products is occurring excessively, becoming emerging issues. Thus, it can cause damage to health, including some allergic reactions and hyperactivity. Therefore, consumers are increasingly demanding and tend to use more natural products, developed from non-toxic resources, hence the emergence of microalgae, as an option for sustainable production and natural sources. Thus, in the market of various sectors of the industry, such as food, beverages, nutritional supplements, and pharmaceutical products, they are implementing bioproducts based on microalgae of the species *Chlorella* sp. and *Spirulina* sp. (Tang et al. 2020). Simultaneously, the species *Dunaliella* and *Arthrospira* (*Spirulina* sp.) also have great potential for numerous commercial uses, as a component for the preparation of various products, not only focusing on the finished product; therefore, the use of microalgae in different sectors of the industry is related to the biomass parameters and structure related to each microalgae (Junior et al. 2020). Thinking in this context, Table 14.2 shows the products and uses of microalgae biomass in different sectors of the industry (Table 14.2).

In fact, microalgae biomass is capable of being used in many industrial sectors. Thus, they are used as a food source, offering a high quality of protein, superior to vegetables. At the same time, microalgae also produce sterols that are used in

Table 14.2 Microalgae products and applications

Microalgae species	Product	Application	References
Arthrospira (*Spirulina*)	Protein, vitamin B_{12}, phycocyanin, carbohydrate	Health food, cosmetics	Chu et al. (2002); Raposo et al. (2013b); Mobin and Alam (2017)
Aphanizomenon flos-aquae	Protein, essential fatty acids, β-carotene	Health food, food supplement	Mobin and Alam (2017)
Phormidium autumnale	Carotenoids, pigments	Food supplement, pharmaceuticals, cosmetics	Rodrigues et al. (2015)
Chlorella zofingiensis	Astaxanthin, colored pigments, biomass, carbohydrate extract	Animal nutrition, health drinks, food supplement	Spolaore et al. (2006)
Chlorella vulgaris	Protein, biomass, carbohydrate extract, ascorbic acid	Health food, food supplement, feeds	Apt and Behrens (1999); Joshi et al. (2018); Mobin and Alam (2017)
Dunaliella salina	Protein, carbohydrate, powders β-carotene, carotenoids, antioxidant	Health food, food supplement, feed	Vonshak (1997); Mobin and Alam (2017); Nascimento et al. (2020a)
Haematococcus pluvialis	Carotenoids, astaxanthin	Health food, food supplement, feed	Nascimento et al. (2020a); Mobin and Alam (2017)
Odontella aurita	Fatty acids, EPA	Pharmaceuticals, cosmetics, anti-inflammatory	Mobin and Alam (2017)
Porphyridium cruentum	Polysaccharides	Pharmaceuticals, cosmetics	Mobin and Alam (2017)
Isochrysis galbana	Fatty acids	Animal nutrition	Lee (1997); Mobin and Alam (2017)
Phaeodactylum tricornutum	Lipids, fatty acids	Nutrition, fuel production	Mobin and Alam (2017)
Lyngbya majuscula	Immune modulators	Pharmaceuticals, nutrition	Mobin and Alam (2017)
Scenedesmus obliquus	Protein, carotenoids	Aquaculture, human nutrition	Mobin and Alam (2017)
Schizochytrium sp.	DHA and EPA	Food, beverage, food supplement	Mobin and Alam (2017)
Crypthecodinium cohnii	DHA	Brain development, infant health, nutrition	Mobin and Alam (2017)
Nannochloropsis oculata	Biomass	Food for larval, juvenile marine fish	Mobin and Alam (2017)
Nannochloropsis sp.	EPA	Food supplement, pharmaceuticals	Mobin and Alam (2017)

Adapted from Rizwan (2018), Mobin and Alam (2017), and Nascimento et al. (2020a)

pharmaceutical sectors as medicine for cardiovascular diseases and microalgae extracts used in cosmetics (Rizwan et al. 2018).

About 200 years ago, the Chinese began to implement microalgae as a food source, given the hunger crisis in their country (Geada et al. 2018). Currently, they are used as food in Asian countries, due to their high nutritional value (Chen et al. 2016; Hong et al. 2015; Um and Kim 2009). According to Tang et al. (2020), a commercial product that uses microalgae in its preparation is M&M chocolate, where *Spirulina* sp. biomass is used as a natural dye. In addition, some establishments produce cooking oil using the technique related to microalgae, generating healthier cooking oil. However, despite efforts to implement microalgae as human food, safety regulations and high manufacturing costs make implementation unfeasible. Consequently, it is in the animal feed trade that microalgae biomass is used, because of its nutritional content and health-related advantages. As a result, biomass is generally marketed in dry or wet mode (Geada et al. 2018; Raja et al. 2016).

In the cosmetics area, the company Daniel Jouvance applies microalgae in the production of its products, due to the potential of microalgae to generate compounds that offer essential benefits for the skin (Tang et al. 2020). In addition, extracts derived from *Spirulina* sp. and *Chlorella* sp. are used as compounds in sunscreens. Therefore, it helps to combat sunburn and ultraviolet radiation (Jha et al. 2017).

In the pharmacology sector, representatives who use algae to develop their products include Agri Life SOM, Phytopharma (India) Limited, Piramal Healthcare, Rincon Pharmaceuticals, and Novo Nordisk India Private Ltd, since microalgae synthesize treated substances for the administration of anticancer drugs. Therefore, microalgae use substances of great importance where it is possible to use them for different uses in medical treatments that can be introduced in the development of new drug technologies for the elimination of diseases, specifically in incurable pathologies (Tang et al. 2020).

Through research related to microalgae so far, they demonstrate their development potential in numerous environmental and industrial applications. However, tests are needed to solve some challenges still encountered for microalgae industrialization technologies, such as high installation and operating costs, microbial contamination of the environment, and light and climate conditions, reaching an imbalance. In that regard, researchers must focus on research related to the processing of microalgae, assessing its potential as a raw material with high promising capacity in biotechnological processes, as well as carrying out tests and technological studies on life cycle assessment, thus obtaining results to prove the economic and sustainable viability concerning microalgae-based processing models (Caporgno and Mathys 2018; Rizwan et al. 2018).

14.5 Conclusions and Future Perspectives

The diversity of microalgal products confirms the excellent performance of these microorganisms in the manufacture of various chemical products, enzymes, and bioactive molecules. The components present in microalgae are precious, with a wide range of applicability, such as human and animal nutrition, biofuels, pharmaceuticals, and cosmetics. In order to obtain these compounds, a more detailed study of cultivation conditions, species, and mainly climatic factors is necessary. Compared to other microorganisms, microalgae have benefits in terms of cost-effectiveness, efficiency, and sustainability.

Microalgae should be exploited among the best strains that produce compounds such as pigments, carbohydrates, lipids, fatty acids, proteins, vitamins, antioxidants, and enzymes. However, parameters that interfere with crop growth, such as climatic factors, must be better analyzed so that the number of desired compounds is produced.

Therefore, the commercial-scale generation of microalgae becomes an economical source, encouraging the manufacture of new products developed and commercialized in the next decade. Until the moment, genetic modifications are being studied to increase the production yield of these microorganisms.

In the near future, new research is expected to endeavor to reduce product losses and thus reduce equipment and energy costs. Also, large-scale processing should be further developed, making processes economically viable and environmentally friendly.

References

Afreen S, Shamsi TN, Baig MA et al (2017) Um romance multicopper oxidase (lacase) de cianobactérias: purificação, caracterização com potencial na descoloração de corante antraquinônico. PLoS One 12:1–20. https://doi.org/10.1371/journal.pone.0175144

Aguilar JGS, Sato HH (2018) Microbial proteases: production and application in obtaining protein hydrolysates. Food Res Int 103:253–262. https://doi.org/10.1016/j.foodres.2017.10.044

Almeida JM, Alnoch RC, Souza EM et al (2020) Metagenomics: is it a powerful tool to obtain lipases for application in biocatalysis? BBA-Proteins Proteom 1868(2):140320. https://doi.org/10.1016/j.bbapap.2019.140320

Apt KE, Behrens PW (1999) Commercial developments in microalgal biotechnology. J Phycol 35 (2):215–226. https://doi.org/10.1046/j.1529-8817.1999.3520215.x

Azzopardi E, Lloyd C, Teixeira SR et al (2016) Clinical applications of amylase: novel perspectives. Surgery 160(1):26–37. https://doi.org/10.1016/j.surg.2016.01.005

Baldev E, MubarakAli D, Ilavarasi A et al (2013) Degradation of synthetic dye, rhodamine B to environmentally non-toxic products using microalgae. Colloids Surf B Biointerfaces 105:207–214. https://doi.org/10.1016/j.colsurfb.2013.01.008

Bekirogullari M, Fragkopoulos IS, Pittman JK et al (2017) Production of lipid-based fuels and chemicals from microalgae: an integrated experimental and model-based optimization study. Algal Res 23:78–87. https://doi.org/10.1016/j.algal.2016.12.015

Bellou S, Baeshen MN, Elazzazy AM et al (2014) Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol Adv 32(8):1476–1493. https://doi.org/10.1016/j.biotechadv.2014.10.003

Bentahar J, Doyen A, Beaulieu L et al (2018) Investigation of β-galactosidase production by microalga *Tetradesmus obliquus* in determined growth conditions. J Appl Phycol 31:301–308. https://doi.org/10.1007/s10811-018-1550-y

Bhalamurugan GL, Valerie O, Mark L et al (2018) Valuable bioproducts obtained from microalgal biomass and their commercial applications: a review. Environ Eng Res 23(3):229–241. https://doi.org/10.4491/eer.2017.220

Bhattacharya M, Goswami S (2020) Microalgae-a green multi-product biorefinery for future industrial prospects. Biocatal Agric Biotechnol 101580. https://doi.org/10.1016/j.bcab.2020.101580

Borowitzka MA (2013) High-value products from microalgae-their development and commercialisation. J Appl Phycol 25(3):743–756. https://doi.org/10.1007/s10811-013-9983-9

Brasil BSAF, Siqueira FG, Salum TFC et al (2017) Microalgae and cyanobacteria as enzyme biofactories. Algal Res 25:76–89. https://doi.org/10.1016/j.algal.2017.04.035

Caporgno MP, Mathys A (2018) Trends in microalgae incorporation into innovative food products with potential health benefits. Front Nutr 5:58. https://doi.org/10.3389/fnut.2018.00058

Chen J, Wang Y, Benemann JR et al (2016) Microalgal industry in China: challenges and prospects. J Appl Phycol 28(2):715–725. https://doi.org/10.1007/s10811-015-0720-4

Chew KW, Yap JY, Show PL et al (2017) Microalgae biorefinery: high value products perspectives. Bioresour Technol 229:53–62. https://doi.org/10.1016/j.biortech.2017.01.006

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chu WL, Phang SM, Miyakawa K et al (2002) Influence of irradiance and inoculum density on the pigmentation of *Spirulina platensis*. Asia Pac J Mol Biol Biotechnol 10(2):109–117

Cripwell R A, Van Zyl WH, Viljoen-Bloom M (2020) Fungal biotechnology: fungal amylases and their applications. Elsevier. doi: https://doi.org/10.1016/b978-0-12-809633-8.21082-0

Davies CM, Apte SC, Peterson SM et al (1994) Plant and algal interference in bacterial β-D-galactosidase and β-D-glucuronidase assays. Appl Environ Microbiol 60:3959–3964. https://doi.org/10.1128/AEM.60.11.3959-3964.1994

Demir BS, Teukel SS (2010) Purification and characterization of lipase from *Spirulina platensis*. J Mol Catal B Enzym 64:123–128. https://doi.org/10.1016/j.molcatb.2009.09.011

Dey PM, Kauss H (1981) α-Galactosidase of *Poterioochromonas malhamensis*. Phytochemistry 20:45–48. https://doi.org/10.1016/0031-9422(81)85216-8

Ejike CE, Collins SA, Balasuriya N et al (2017) Prospects of microalgae proteins in producing peptide-based functional foods for promoting cardiovascular health. Trends Food Sci Tech 59:30–36. https://doi.org/10.1016/j.tifs.2016.10.026

Ellatif SA, El-Sheekh MM, Senousy HH (2020) Role of microalgal ligninolytic enzymes in industrial dye decolorization. Int J Phytoremediation:1–12. https://doi.org/10.1080/15226514.2020.1789842

Enamala MK, Enamala S, Chavali M et al (2018) Production of biofuels from microalgae-a review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renew Sust Energ Rev 94:49–68. https://doi.org/10.1016/j.rser.2018.05.012

Eriksen NT (2008) The technology of microalgal culturing. Biotechnol Lett 30(9):1525–1536. https://doi.org/10.1007/s10529-008-9740-3

Erpel F, Restovic F, Arce-Johnson P (2016) Development of phytase-expressing *Chlamydomonas reinhardtii* for monogastric animal nutrition. BMC Biotechnol 16(1):1–7. https://doi.org/10.1186/s12896-016-0258-9

Fabregas J, Herrero C (1990) Vitamin content of four marine microalgae. Potential use as source of vitamins in nutrition. J Ind Microbiol 5(4):259–263. https://doi.org/10.1007/BF01569683

Fagundes MB, Vendruscolo RG, Wagner R (2020) In: Jacob-Lopes E, Maroneze MM, Queiroz MI, Zepka LQ (eds) Sterols from microalgae. Handbook of microalgae-based processes and products. Academic Press. p 573–596. doi:https://doi.org/10.1016/b978-0-12-818536-0.00021-x.

Fernandes BD, Mota A, Teixeira JA et al (2015) Continuous cultivation of photosynthetic microorganisms: approaches, applications and future trends. Biotechnol Adv 33 (6):1228–1245. https://doi.org/10.1016/j.biotechadv.2015.03.004

Geada P, Vasconcelos V, Vicente A et al (2017) In: Rastogi RP, Madamwar D, Pandey A (eds) Microalgal biomass cultivation. Algal green chemistry. Elsevier. p 257-284. doi: 10.1016/B978-0-444-63784-0.00013-8.

Geada P, Rodrigues R, Loureiro L et al (2018) Electrotechnologies applied to microalgal biotechnology-applications, techniques and future trends. Renew Sust Energ Rev 94:656–668. https://doi.org/10.1016/j.rser.2018.06.059

Gimpel JA, Henríquez V, Mayfield SP (2015) In the metabolic engineering of eukaryotic microalgae: potential and challenges come with great diversity. Front Microbiol 6:1376. https://doi.org/10.3389/fmicb.2015.01376

Giordano M, Wang Q (2018) In: Vaz S Jr (ed) Microalgae for industrial purposes, Biomass and green chemistry. Springer, Cham, pp 133–167. https://doi.org/10.1007/978-3-319-66736-2_6

Girard J-M, Roy M-L, Hafsa MB et al (2014) Mixotrophic cultivation of green microalgae *Scenedesmus obliquus* on cheese whey permeate for biodiesel production. Algal Res 5:241–248. https://doi.org/10.1016/j.algal.2014.03.002

Godet S, Herault J, Pencreach G et al (2012) Isolation and analysis of a gene from the marine microalga *Isochrysis galbana* that encodes a lipase-like protein. J Appl Phycol 24:1547–1553. https://doi.org/10.1007/s10811-012-9815-3

Graziani G, Schiavo S, Nicolai MA et al (2013) Microalgae as human food: chemical and nutritional characteristics of the thermo-acidophilic microalga *Galdieria sulphuraria*. Food Funct 4(1):144–152. https://doi.org/10.1039/C2FO30198A

Handa V, Sharma D, Kaur A et al (2020) Biotechnological applications of microbial phytase and phytic acid in food and feed industries. Biocatal Agri Biotechnol 25:101600. https://doi.org/10.1016/j.bcab.2020.101600

Hess SK, Lepetit B, Kroth PG et al (2018) Production of chemicals from microalgae lipids–status and perspectives. Eur J Lipid Sci Tech 120(1):1700152. https://doi.org/10.1002/ejlt.201700152

Ho SH, Huang SW, Chen CY et al (2013) Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. Bioresour Technol 135:191–198. https://doi.org/10.1016/j.biortech.2012.10.015

Hong JW, Jo SW, Yoon HS (2015) Research and development for algae-based technologies in Korea: a review of algae biofuel production. Photosynth Res 123(3):297–303. https://doi.org/10.1007/s11120-014-9974-y

Hubert F, Poisson L, Loiseau C et al (2017) Lipids and lipolytic enzymes of the microalga *Isochrysis galbana*. Oilseeds fats crop lipids 24: D407. Doi: 10.1051 / ocl / 2017023

Husain Q (2010) β galactosidases and their potential applications: a review. Crit Rev Biotechnol 30:41–62. https://doi.org/10.3109/07388550903330497

Jacob-Lopes E, Maroneze MM, Deprá MC et al (2019) Bioactive food compounds from microalgae: an innovative framework on industrial biorefineries. Curr Opin Food Sci 25:1–7. https://doi.org/10.1016/j.cofs.2018.12.003

Japar AS, Takriff MS, Yasin NHM (2017) Harvesting microalgal biomass and lipid extraction for potential biofuel production: a review. J Environ 5(1):555–563. https://doi.org/10.1016/j.jece.2016.12.016

Jha D, Jain V, Sharma B et al (2017) Microalgae based pharmaceuticals and nutraceuticals: an emerging field with immense market potential. Chembioeng Rev 4(4):257–272. https://doi.org/10.1002/cben.201600023

Joshi S, Kumari R, Upasani VN (2018) Applications of algae in cosmetics: an overview. Int J Innov Res Sci Eng Technol 7(2):1269. https://doi.org/10.15680/IJIRSET.2018.0702038

Junior WGM, Gorgich M, Corrêa PS et al (2020) Microalgae for biotechnological applications: cultivation, harvesting and biomass processing. Aquac 735562. https://doi.org/10.1016/j.aquaculture.2020.735562

Klanbut K, Peerapornpisarn Y, Khanongnuch C et al (2002) Phytase from some strains of thermophilic blue-green algae. J Phycol S2:57–60

Koller M, Salerno A, Tuffner P et al (2012) Characteristics and potential of micro algal cultivation strategies: a review. J Clean Prod 37:377–388. https://doi.org/10.1016/j.jclepro.2012.07.044

Koller M, Muhr A, Braunegg G (2014) Microalgae as versatile cellular factories for valued products. Algal Res 6:52–63. https://doi.org/10.1016/j.algal.2014.09.002

Kombrink E, Weober G (1980) Identification and subcellular localization of starch-metabolizing enzymes in the green alga *Dunaliella marina*. Planta 149:130–137. https://doi.org/10.1007/BF00380873

Lafarga T, Fernández-Sevilla JM, González-López C et al (2020) *Spirulina* for the food and functional food industries. Food Res Int 109356. https://doi.org/10.1016/j.foodres.2020.109356

Lee YK (1997) Commercial production of microalgae in the Asia-Pacific rim. J Appl Phycol 9 (5):403–411. https://doi.org/10.1023/A:1007900423275

Levasseur W, Perré P, Pozzobon V (2020) A review of high value-added molecules production by microalgae in light of the classification. Biotechnol Adv 107545. https://doi.org/10.1016/j.biotechadv.2020.107545

Levi C, Gibbs M (1984) Starch degradation in synchronously grown *Chlamydomonas reinhardtii* and characterization of the amylase. Plant Physiol 74:459–463. https://doi.org/10.1104/pp.74.3.459

Li X, Li S, Liang X et al (2020) Applications of oxidases in modification of food molecules and colloidal systems: laccase, peroxidase and tyrosinase. Trends Food Sci Tech 103:78–93. https://doi.org/10.1016/j.tifs.2020.06.014

Lockau W, Massalsky B, Dirmeier A (1988) Purification and partial characterization of a calcium-stimulated protease from the cyanobacterium, *Anabaena variabilis*. Eur J Biochem 172:433–438. https://doi.org/10.1111/j.1432-1033.1988.tb13906.x

Maeda Y, Yoshino T, Matsunaga T et al (2018) Marine microalgae for production of biofuels and chemicals. Curr Opin Biotech 50:111–120. https://doi.org/10.1016/j.copbio.2017.11.018

Manoj BS, Sushma CM, Karosiya A (2018) Western Ghats terrestrial microalgae serve as a source of amylase and antioxidants enzymes. J Pharmacogn Phytochem 7:1555–1560

Markou G, Nerantzis E (2013) Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. Biotechnol Adv 31(8):1532–1542. https://doi.org/10.1016/j.biotechadv.2013.07.011

Markou G, Angelidaki I, Georgakakis D (2012) Microalgal carbohydrates: an overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. Appl Microbiol Biot 96(3):631–645. https://doi.org/10.1007/s00253-012-4398-0

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renew Sust Energ Rev 14(1):217–232. https://doi.org/10.1016/j.rser.2009.07.020

Matos ÂP (2017) The impact of microalgae in food science and technology. J Am Oil Chem Soc 94 (11):1333–1350. https://doi.org/10.1007/s11746-017-3050-7

Medina JDC, Woiciechowski AL, Guimarães LRC et al (2016) In: Pandey a, Negi S, Soccol CR (eds) peroxidases. Current developments in biotechnology and bioengineering: production, isolation and purification of industrial products. Elsevier, p. 217-232. doi: https://doi.org/10.1016/B978-0-444-63662-1.00010-5

Mehta D, Satyanarayana T (2016) Bacterial and archaeal α-amylases: diversity and amelioration of the desirable characteristics for industrial applications. Front Microbiol 7:1129. https://doi.org/10.3389/fmicb.2016.01129

Mobin S, Alam F (2017) Some promising microalgal species for commercial applications: a review. Energy Procedia 110:510–517. https://doi.org/10.1016/j.egypro.2017.03.177

Mohanan N, Satyanarayana T (2019) Amylases. In: Schmidt TM (ed) Encyclopedia of microbiology. Fourth, p. 107-126

Moradi S, Ziaei R, Foshati S et al (2019) Effects of *spirulina* supplementation on obesity: a systematic review and meta-analysis of randomized clinical trials. Complement Ther Med 47:102211. https://doi.org/10.1016/j.ctim.2019.102211

de Morais WGJ, Gorgich M, Corrêa PS et al (2020) Microalgae for biotechnological applications: cultivation, harvesting and biomass processing. Aquac 735562. https://doi.org/10.1016/j.aquaculture.2020.735562

Murphy CD, Moore RM, White RL (2000) Peroxidases from marine microalgae. J Appl Phycol 12:507–513. https://doi.org/10.1023/A:1008154231462

Nanni B, Balestreri E, Dainese E et al (2001) Characterization of a specific Phycocyanin hydrolysing protease purified from *Spirulina platensis*. Microbiol Res 156:259–266. https://doi.org/10.1078/0944-5013-00110

Nascimento TC, Cazarin CBB, Maróstica JMR et al (2020a) Microalgae carotenoids intake: influence on cholesterol levels, lipid peroxidation and antioxidant enzymes. Food Res Int 128:108770. https://doi.org/10.1016/j.foodres.2019.108770

Nascimento TC, Nass PP, Fernandes AS et al (2020b) Exploratory data of the microalgae compounds for food purposes. Data Brief 29:105182. https://doi.org/10.1016/j.dib.2020.105182

Naumoff DG (2011) Hierarchical classification of glycoside hydrolases. Biochem Mosc 76:622–635. https://doi.org/10.1134/s0006297911060022

Niven GW (1995) The characterization of two aminopeptidase activities from the cyanobacterium *Anabaena flosaquae*. Biochim Biophys Acta Protein Struct Mol Enzymol 1253:193–198. https://doi.org/10.1016/0167-4838(95)00175-0

Nur MMA, Buma AG (2019) Opportunities and challenges of microalgal cultivation on wastewater, with special focus on palm oil mill effluent and the production of high value compounds. Waste Biomass Valorization 10(8):2079–2097. https://doi.org/10.1007/s12649-018-0256-3

Oesterhelt C, Vogelbein S, Shrestha RP et al (2008) The genome of the thermoacidophilic red microalga *Galdieria sulphuraria* encodes a small family of secreted class III peroxidases that might be involved in cell wall modification. Planta 227:353–362. https://doi.org/10.1007/s00425-007-0622-z

Olguín EJ (2012) Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. Biotechnol Adv 30(5):1031–1046. https://doi.org/10.1016/j.biotechadv.2012.05.001

Otto B, Schlosser D, Reisser W (2010) First description of a laccase-like enzyme in soil algae. Arch Microbiol 192:759–768. https://doi.org/10.1007/s00018-009-0169-1

Otto B, Beuchel C, Liers C et al (2015) Laccase-like enzyme activities from chlorophycean green algae with potential for bioconversion of phenolic pollutants. FEMS Microbiol Lett 362:1–8. https://doi.org/10.1093/femsle/fnv072

Overbaugh JM, Fall R (1982) Detection of glutathione peroxidases in some microalgae. FEMS Microbiol Lett 13:371–375. https://doi.org/10.1111/j.1574-6968.1982.tb08290.x

Overbaugh JM, Fall R (1985) Characterization of a selenium-independent glutathione peroxidase from *Euglena gracilis*. Plant Physiol 77:437–442. https://doi.org/10.1104/pp.77.2.437

Patias LD, Maroneze MM, Siqueira SF et al (2018) Single-cell protein as a source of biologically active ingredients for the formulation of Antiobesity foods. Alternative and Replacement Foods:317–353. https://doi.org/10.1016/b978-0-12-811446-9.00011-3

Patil K, Mahajan RT (2016) Enzymatic study of fresh water macro and micro algae isolated from Jalgaon, Maharashtra. Int J Pharm Bio Sci 7:207–215. https://doi.org/10.4172/2155-952X.C1.068

Patil KJ, Mahajan RT, Mahajan SR et al (2001) Enzyme profile of fresh water uncultured algae belonging to Bhusawal region, Maharashtra. J Chem biosphere 2(1): 33-28. Doi: https://doi.org/10.20546/ijcrbp.2016.301.013

Pignolet O, Jubeau S, Vaca-Garcia C et al (2013) Highly valuable microalgae: biochemical and topological aspects. J Ind Microbiol Biot 40(8):781–796. https://doi.org/10.1007/s10295-013-1281-7

Pragya N, Pandey KK, Sahoo PK (2013) A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renew Sust Energ Rev 24:159–171. https://doi.org/10.1016/j.rser.2013.03.034

Rahman KM (2020) In: Alam MA, Jing-Liang X, Zhongming W (eds) Food and high value products from microalgae: market opportunities and challenges. Microalgae biotechnology for food, health and high value products. Springer, Singapore, . p 3-27. doi: 10.1007/978-981-15-0169-2_1

Raja R, Hemaiswarya S, Ganesan V et al (2016) Recent developments in therapeutic applications of cyanobacteria. Crit Rev Microbiol 42(3):394–405. https://doi.org/10.3109/1040841X.2014.957640

Raposo MFJ, de Morais RMSC, de Morais AMMB (2013a) Health applications of bioactive compounds from marine microalgae. Life Sci 93(15):479–486. https://doi.org/10.1016/j.lfs.2013.08.002

Raposo MFDJ, De Morais RMSC, Bernardo de Morais AMM (2013b) Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs 11(1):233–252. https://doi.org/10.3390/md11010233

Rizwan M, Mujtaba G, Memon SA et al (2018) Exploring the potential of microalgae for new biotechnology applications and beyond: a review. Renew Sust Energ Rev 92:394–404. https://doi.org/10.1016/j.rser.2018.04.034

Rocha CM, Genisheva Z, Ferreira-Santos P et al (2018) Electric field-based technologies for valorization of bioresources. Bioresour Technol 254:325–339. https://doi.org/10.1016/j.biortech.2018.01.068

Rodrigues DB, Menezes CR, Mercadante AZ et al (2015) Bioactive pigments from microalgae *Phormidium autumnale*. Food Res Int 77:273–279. https://doi.org/10.1016/j.foodres.2015.04.027

Savvidou MG, Sotiroudis TG, Kolisis FN (2016) Cell surface and cellular debris-associated heat-stable lipolytic enzyme activities of the marine alga *Nannochloropsis oceanica*. Biocatal Biotransformation 34:24–32. https://doi.org/10.1080/10242422.2016.1212843

Sharma KM, Kumar R, Panwar S et al (2017) Microbial alkaline proteases: optimization of production parameters and their properties. J Genet Eng Biotechnol 15(1):115–126. https://doi.org/10.1016/j.jgeb.2017.02.001

Sharma A, Ahluwalia O, Tripathi AD et al (2020) Phytases and their pharmaceutical applications: mini-review. Biocatal Agric Biotechnol 23:101439. https://doi.org/10.1016/j.bcab.2019.101439

da Silva Vaz B, Moreira JB, de Morais MG et al (2016) Microalgae as a new source of bioactive compounds in food supplements. Curr Opin Food Sci 7:73–77. https://doi.org/10.1016/j.cofs.2015.12.006

Silva PEC, Souza FASD, Barros RC et al (2017) Enhanced production of fibrinolytic protease from microalgae *Chlorella vulgaris* using glycerol and corn steep liquor as nutrient. Ann Microbiol Res 1: 9-19. Doi: 10.36959/958/564

Spier MR, Peron-schlosser B, Paludo LC et al (2020) Microalgae as enzymes biofactories. In: Jacob-Lopes E, Queiroz MI, Maroneze MM, Zepka LQ (eds) Handbook of microalgae-based processes and products. Academic, New York, pp 687–706. https://doi.org/10.1016/B978-0-12-818536-0.00025-7

Spolaore P, Joannis-Cassan C, Duran E et al (2006) Commercial applications of microalgae. J Biosci Bioeng 101(2):87–96. https://doi.org/10.1263/jbb.101.87

Strohmeier U, Gerdes C, Lockau W (1994) Proteolysis in heterocyst-forming cyanobacteria: characterization of a further enzyme with trypsin-like specificity, and of a prolyl endopeptidase from *Anabaena variabilis*. Z Naturforsch C J Biosci 49:70–78. https://doi.org/10.1515/znc-1994-1-212

Suwal S, Bentahar J, Marciniak A et al (2019) Evidence of the production of galactooligosaccharide from whey permeate by the microalgae *Tetradesmus obliquus*. Algal Res 39:101470. https://doi.org/10.1016/j.algal.2019.101470

Tang DYY, Khoo KS, Chew KW et al (2020) Potential utilization of bioproducts from microalgae for the quality enhancement of natural products. Bioresour Technol 304:122997. https://doi.org/10.1016/j.biortech.2020.122997

Um BH, Kim YS (2009) A chance for Korea to advance algal-biodiesel technology. J Ind Eng Chem 15(1):1–7. https://doi.org/10.1016/j.jiec.2008.08.002

Vidya CH, Kumar BS, Chinmayee CV et al (2020) Purification, characterization and specificity of a new GH family 35 galactosidase from *Aspergillus awamori*. Int J Biol Macromol 156:885–895. https://doi.org/10.1016/j.ijbiomac.2020.04.013

Vilchez C, Garbayo I, Lobato MV et al (1997) Microalgae-mediated chemicals production and wastes removal. Enzyme Microb Technol 20(8):562–572. https://doi.org/10.1016/S0141-0229(96)00208-6

Vonshak A (1997) Microalgal biotechnology: new development in production facilities and products. In 2: Asia-Pacific marine biotechnology conference and 3. Asia-Pacific conference on algal biotechnology, Phuket (Thailand)

Yada E, Nagata H, Noguchi Y et al (2005) An arginine specific protease from *Spirulina platensis*. Mar Biotechnol 7:474–480. https://doi.org/10.1007/s10126-004-4115-9

Yen HW, Hu IC, Chen CY et al (2013) Microalgae-based biorefinery-from biofuels to natural products. Bioresour Technol 135:166–174. https://doi.org/10.1016/j.biortech.2012.10.099

Yong SK, Lim BH, Saleh S et al (2016) Optimisation, purification and characterization of extracellular lipase from *Botryococcus sudeticus* (UTEX 2629). J Mol Catal B Enzym 126:99–105. https://doi.org/10.1016/j.molcatb.2016.02.004

Zanette CM, Mariano AB, Yukawa YS et al (2019) Microalgae mixotrophic cultivation for β-galactosidase production. J Appl Phycol 31:1597. https://doi.org/10.1007/s10811-018-1550-y

Zepka LQ, Jacob-Lopes E, Goldbeck R et al (2010) Nutritional evaluation of single-cell protein produced by *Aphanothece microscopica Nägeli*. Bioresour Technol 101(18):7107–7111. https://doi.org/10.1016/j.biortech.2010.04.001

Zepka LQ, Jacob-Lopes E, Roca M (2019) Catabolism and bioactive properties of chlorophylls. Curr Opin Food Sci 26:94–100. https://doi.org/10.1016/j.cofs.2019.04.004

Zhao R, Zhao R, Tu Y et al (2018) A novel α-galactosidase from the thermophilic probiotic *Bacillus coagulans* with remarkable protease-resistance and high hydrolytic activity. PLoS One 13: e0197067. https://doi.org/10.1371/journal.pone.0197067

Zhu LD, Hiltunen E (2016) Application of livestock waste compost to cultivate microalgae for bioproducts production: a feasible framework. Renew Sust Energ Rev 54:1285–1290. https://doi.org/10.1016/j.rser.2015.10.093

6 REFERÊNCIAS

ABRAGAMSSON, I.; RODRIGUEZ-MEIZOSO, C.; TURNER. Supercritical fluid extraction of lipids from linseed with on-line evaporative light scattering detection, **Analytica Chimica Acta**, 853, 320-327, 2015.

ALBUQUERQUE, C.D. Estudo da cinética de produção de carotenoides de Sporobolomyces ruberrimus e de técnicas de extração de pigmentos. Dissertação. (Mestrado em Engenharia de Alimentos) – Universidade Federal de Santa Catarina, Florianópolis-SC. 177p**,** 2015.

BENNEDSEN, M., WANG, X., WILLIÉN, R., WADSTROM, T., ANDERSEN, L.P. (2000). Treatment of *H. pylori* infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. **Immunology letters**, 70, 185-189, 2000.

BJØRLAND, T., LIAAEN-JENSEN, S., THRONDSEN, J. Carotenoids of the marine *chrysophyte Pelagococcus subviridis*. **Phytochemistry**, 28(12), 3347–3353, 1989.

BOROWITZKA, M.A. Biology of Microalgae. In: Microalgae in Health and Disease Prevention. Edited by Levine IA, Fleurence J. Academic Press; 23-72, 2018.

BOROWITZKA, M.A. Chapter 9 - Microalgae in Medicine and Human Health: A Historical Perspective. **Microalgae in Health and Disease Prevention**, 195-210, 2018.

BOROWITZKA, M.A. High-value products from microalgae-their development and commercialization. **Journal of Applied Phycology**, 25, 743-756, 2013.

BRASIL, B.S.A.F., SILVA, F.C.P., SIQUEIRA. Microalgae biorefineries: The Brazilian scenario in perspective. **New Biotechnology**,39, 90-98, 2017.

BRITTON, G. Chapter 10: functions of intact carotenoids. In: BRITTON, G., PFANDER,H., LIAAEN-JENSEN, S. Carotenoids. Volume 4: natural functions. **Birkhäuser Basel,** 189–212, 2008b.

BRITTON, G., LIAAEN-JENSEN, S., PFANDER, H. Carotenoids: Handbook. 1st ed. Basel, Switzerland:Springer Basel AG; 2004. p. 646p.

BUSINESS COMMUNICATIONS COMPANY. The Global Market for Carotenoids - FOD025E http://www.bccresearch.com/market-research/food-and-beverage/carotenoids-global marketreport-fod025e.html (2015).acessado 22.02.19.

BUSTAMANTE, A., MASSON, L., VELASCO, J.,VALLE, J.M., ROBERT, P. Microencapsulation of H. pluvialis oleoresins with different fatty acid composition: Kinetic stability of astaxanthin and alpha-tocopherol. **Food Chemistry** 190, 1013–1021, 2016.

CHA, K., KOO, S., LEE, D. Antiproliferative effects of carotenoids extracted from *Chlorella ellipsoidea* and *Chlorella vulgaris on* human colon cancer cells. **Journal of Agricultural and Food Chemistry**, 56, 10521–10526, 2008.

CHACON-LEE, T. L.; GONZALEZ-MARINO, G. E. Microalgae for "healthy" foods: possibilities and challenges. Comprehensive Reviews. **Food Science and Food Safety**, 9, 655–675, 2010.

CHEN, C.; JESISCA.; HSIEH, C.; LEE, D.; CHANG, C.; CHANG, J. Production, extraction and stabilization of lutein from microalga *Chlorella sorokiniana* MB-1. **Bioresource Technology**, 200, 500-505, 2016.

CHEN, F. High cell density culture of microalgae in heterotrophic growth. **Trends in Biotechnology**. 14, 421–426, 1996.

CHEN, G. Q.; CHEN, F. Growing phototrophic cells without light. **Biotechnology Letters**, v. 28, p. 607-616, 2006.

CHEN, Y-H, WALKER, T.H. Fed-batch fermentation and supercritical fluid extraction of heterotrophic microalgal *Chlorella* protothecoides lipids. **Bioresource Technology**, 114, 512–517,2012.

CHEW, K.W., YAP, J.Y., SHOW, P.L., SUAN, N.H., JUAN, J.C., LING, T.C., LEE, D.J., CHANG, J.S. Microalgae biorefinery: High value products perspectives. **Bioresource Technology**, 229, 53-62, 2017.

CHIU, P.; SOONG, K.; CHEN, C.N. Cultivation of two thermotolerant microalgae under tropical conditions: Influences of carbon sources and light duration on biomass and lutein productivity in four seasons. **Bioresource Technology**, 212, 190-198, 2016.

CHIU, S.Y., KAO, C.Y., CHEN, C.H., KUAN, T.C., ONG, S.C., LIN, C.S. Reduction of CO2 by a high-density culture of *Chlorella sp* in a semicontinuous photobioreactor. **Bioresource Technology**, 99, 3389–3396, 2008.

CÓRDOVA, O., SANTIS, J., RUIZ-FILLIPI, G., ZUÑIGA, M.E., FERMOSO, F.G., CHAMY, R. Microalgae digestive pretreatment for increasing biogas production. **Renewable and Sustainable Energy Reviews**. 82, 2806-2813, 2017.

D'ALESSANDRO, E. B.; ANTONIOSI FILHO, N. R. Concepts and studies on lipid and pigments of microalgae: A review. **Renewable and Sustainable Energy Reviews**, 58, 832-841, 2016.

DI LENA, G., CASINI I., LUCARINI, M., LOMBARDI-BOCCIA, G. Carotenoid profiling of five microalgae species from large-scale production. **Food Research International**, *In Press*, 2018.

DUFOSSÉ, L. (2016). Current and Potential Natural Pigments From Microorganisms (Bacteria, Yeasts, Fungi, Microalgae). **Woodhead Publishing**, 337-354, 2016.

EROGLU, A., HARRISON, E.H. Carotenoid metabolism in mammals, including man: formation, occurrence, and function of apocarotenoids. **Journal of Lipid Research**, 54, 1719-1730, 2013.

FAKHRI, S., DARGAHI, L., ABBASZADEH, F., JORJANI, M. Astaxanthin attenuates neuroinflammation contributed to the neuropathic pain and motor dysfunctionfollowing compression spinal cord injury. **Brain Research Bulletin**, 143, 217, 224, 2018.

FAY, P. *The Blue-Greens (Cyanophyta-Cyanobacteria)*, 5th Edn. London: Edward Arnold Publishers, 88. Studies in Biology no. 160, 1983.

FRANCISCO, E. C., FRANCO, T. T., WAGNER, R., and JACOB-LOPES, E. (2014). Assessment of different carbohydrates as exogenous carbon source in cultivation of cyanobacteria. **Bioprocess and Biosystems Engineering**. 37, 1497–1505.

FRASER, P.D., BRAMLEY, P.M. The biosynthesis and nutritional uses of carotenoids. **Progress in Lipid Research**. 43:228–265, 2004.

GARCÍA-GONZÁLEZ, M.; MORENO, J.; MANZANO, J.C.; FLORENCIO, F.J.; GUERRERO, M.G. Production of Dunaliella salina biomass rich in 9-cis-ß-carotene and lutein in a closed tubular photobioreactor. **Journal of Biotechnology,** 115, 81-90, 2005.

GAYATHRI, S., RAJASREE, S.R.R., KIRUBAGARAN, R., ARANGANATHAN, L.; SUMAN, T.Y. Spectral characterization of ß, e-carotene-3, 3'-diol (lutein) from marine microalgae *Chlorella salina*. **Renewable Energy**, 98, 78-93, 2016.

GERARDO, M. L.; VAN DEN HENDE, S.; VERVAEREN, H.; COWARD, T.; SKILL, S. C. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. **Algal Research**, 11, 248-262, 2015.

GERKEN, H., DONOHOE, B., KNOSHAUG, E. Enzymatic cell wall degradation of *Chlorella vulgaris* and other microalgae for biofuels production. **Planta**.237, 239–253, 2013.

GILDENHOFF, N., AMARIE, S., GUNDERMANN, K., BEER, A., BUCHEL, C., WACHTVEITL, J. Oligomerization and pigmentation dependent excitation energy transfer in fucoxanthin–chlorophyll proteins. **Biochimica et Biophysica Acta (BBA) - Bioenergetics**, 1797(5), 543–549, 2010.

GOIRIS, k., MUYLAERT, K., FRAEYE, I., FOUBRT, M., BRABATER, J., COOMAN, L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. **Journal of Applied Phycology**, 24, 1477-1486, 2012.

GONG, M., BASSI, A. Carotenoids from microalgae: A review of recent developments. **Biotechnology Advances**, 34, 1396-1412, 2016.

GRUDZINSKI, W., KRZEMINSKA, I., LUCHOWSKI, R., NOSALEWCZ, A.; GRUSZECKI, W. L. Strong-light-induced yellowing of green microalgae *Chlorella*: A study on molecular mechanisms of the acclimation response. **Algal Research**, 16, 245-254, 2016.

GUEDES, A., AMARO, H., MALCATA, F. Microalgae as Sources of Carotenoids. **Marine drugs**, 9,625–644, 2011.

GULDHE, A., ANSARI, F.A., SINGH, P., BUS, F. Heterotrophic cultivation of microalgae using aquaculture wastewater: A biorefinery concept for biomass production and nutrient remediation. **Ecological Engineerin.** 99, 47-53, 2017.

HAN, D. X., LI, Y. T., & HU, Q. Astaxanthin in microalgae: pathways, functions and biotechnological implications. **Algae research,** 28(2), 131–147, 2013.

HERRERO, M., IBÁÑEZ, E. Green processes and sustainability: An overview on the extraction of high added-value products from seaweeds and microalgae. **The Journal of Supercritical Fluids**, 96, 211-216, 2015.

HU, J., NAGARAJAN, D., ZHANG, Q., CHANG, J.-S., LEE, D.J. Heterotrophic cultivation of microalgae for pigment production: A review. **Biotechnology Advances**, 36(1), 54–67, 2018.

JACOB-LOPES, E. et al. Characteristics of thin-layer drying of the cyanobacterium Aphanothece microscopica Nägeli. **Chemical Engineering and Processing**, v. 46, p. 63–69, 2007.

JACOB-LOPES, E., MARONEZE, M.M., DEPRÁ, M.C., SARTORI, R.B., DIAS, R.R., ZEPKA, L.Q. Bioactive food compounds from microalgae: An innovative framework on industrial biorefineries. **Current opinion in Food Science,** 25, 1-7, 2019.

JACOB-LOPES, E.; LACERDA, L. M. C. F.; FRANCO, T. T. Biomass production and carbon dioxide fixation by Aphanothece microscopica Nägeli in a bubble column photobioreactor. **Biochemical Engineering Journal**, v.40, p. 27–34, 2008.

JACOB-LOPES, E.; QUEIROZ, M. I.; ZEPKA, L. Q.; NETTO, F. M. Protein characterisation of the *Aphanothece Microscopica Nägeli* cyanobacterium cultivated in parboiled rice effluent. **Food Science and Technology**, v. 26, p. 482–488, 2006.

JACOB-LOPES, E.; REVAH, S.; SHIRAI, K.; HERNANDEZ, S.; FRANCO, T. T. Development of operational strategies to remove carbon dioxide in photobioreactors. **Chemical Engineering Journal**, v. 153, p. 120–126, 2009.

JACOB-LOPES, E.; SCOPARO, C. H. G.; QUEIROZ, M. I.; FRANCO, T. T. Biotransformations of carbon dioxide in photobioreactors. **Energy Conversion and Management**, v. 51, p. 894–900, 2010.

JAIME, L., RODRÍGUEZ-MEIOZOSO, I., CIFUENTES, A., SANTOYO, S., SUAREZ, S., IBÁÑEZ, E., SEÑORANS, F.J. (2010). Pressurized liquids as an alternative process to

antioxidante carotenoids' extraction from Haematococcus pluvialis microalgae**. LWT-Food Science and Technology**, 43, 105-112, 2010.

KAMOSHITA, M., TODA, E., OSADA, H., NARIMATSU, T., KOBAYASHI, S., TSUBOTA, K., OZAWA, Y. Lutein acts via multiple antioxidant pathways in the photo-stressed retina. **Nature Scientific Reports**, 6(1), 2016.

KEBEBE, B.T., GRAUWET, T., MAGPUSAO, J., PALMERS, S., MICHIELS, C., HENDRICKX, M., LOEY, A.V. An integrated fingerprinting and kinetic approach to accelerated shelf-life testing of chemical changes in thermally treated carrot puree. **Food Chemistry**, 179, 94-102, 2015.

KHAN, S.A., RASHMI, HUSSAIN, M.Z., PRASAD, S., BANERJEE, U.C. Prospects of biodiesel production from microalgae in India. **Renewable and Sustainable Energy Reviews** 13, 2361–2372,2009.

KIM, K.; JUNG, J.; SHIN, H.; CHOI, S.; KIM. D.; BAI, S. C.; CHANG, Y. K.; HAN, J. Harvesting of *Scenedesmus obliquus* using dynamic filtration with a perforated disk. **Journal of Membrane Science**, 517, 14-20, 2016.

KOSAKOWSKA, A., LEWANDOWKA, J., STÓN, J., BURKIEWICZ, K. Qualitative and quantitative composition of pigments in *Phaeodactylum tricornutum (Bacillariophyceae*) stressed by iron. **BioMetals**, 17(1), 45–52, 2004.

KULKARNI, S., *NIKOLOV,* Z. Process for selective extraction of pigments and functional proteins from *Chlorella vulgaris*. **Algal Research**. 35, 185-193, 2018.

LEE, E., JALALIZADEH, M., & ZHANG, Q. Growth kinetic models for microalgae cultivation: A review. **Algal Research**, 12, 497–512, 2015.

LEYA, T.; RAHN, A.; LUTZ, C.; REMIAS, D. Response of arctic snowand permafrost algae to high light and nitrogen stress by changes in pigment compositionand applied aspects for biotechnology. **FEMS Microbiology Letters**, 67, 432-443, 2009.

LI, H.B., CHENG, K.W., WONG, C.C., FAN, K.W., CHEN, F., JIANG, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. **Food Chemistry**, 102,771–776, 2007.

LIN, J.-H., LEE, D.-J., CHANG J.-S. Lutein production from biomass: Marigold flowers versus microalgae. **Bioresource Technology**, 184, 421–428, 2015.

LIU, J., & CHEN, F. Biology and Industrial Applications of *Chlorella*: Advances and Prospects. **Advances in Biochemical Engineering/Biotechnology**, 1–35, 2014.

LIU, Y.S.; WU, J.Y. Optimization of cell growth and carotenoid production of Xanthophyllomyces dendrorhous through statistical experiment design. **Journal of Biochemical Engineering**, v.36, p. 182–189, 2007.

LORETO, C., ROSALES, N., BERMÚDEZ, J., MORLAES, E. Produccíon de pigmentos y protéınas de la cianobacteria *Anabaena PCC 7120* en relacíon a la concentracíon de nitrógeno e irradiancia. **Gayana Botánica**, 60(2), 83–9, 2003.

LOURENÇO, S. O. Cultivo de Microalgas Marinhas: Princípios e Aplicações. São Carlos: **RiMa**, 606p., 2006.

MA, N.L.; THE, K.Y.; LAM, S.S.; KABEN, A.M.; CHA, T.S. Optimization of cell disruption methods for efficient recovery of bioactive metabolites via NMR of three freshwater microalgae (chlorophyta). **Bioresource Technology**, v. 190, p. 536-542, 2015.

MANAYI, A., ABDOLLAHI, M., RAMAN, T., NEBAVI, S.T., HABTEMARIAM, S., DAGLIA, M., NABAVI, A.M. Lutein and cataract: from bench to bedside. **Critical Reviews in Biotechnology,** 8551, 1-11. 2015.

MARONEZE, M. M. et al. The role of photoperiods on photobioreactors - A potential strategy to reduce costs. **Bioresource Technology**, v.219, p.493–499, 2016.

MORENO-GARCIA, L., ADJALLÉ, K., BARNABÉ, S., RAGHAVAN, G.S.V. Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. **Renewable and Sustainable Energy Reviews,** 76,493-506,2017.

NIRANJANA, R., GAYATHRI, R., NIMISH MOL, S., SUGAWARA, T., HIRATA, T., MIYASHITA, K., GANESAN, P. Carotenoids modulate the hallmarks of cancer cells. **Journal of Functional Foods**, 18, 968-985, 2015.

ONG, S-C., KAO, C-Y., CHIU, S-Y., TSAI, M-T., LIN, C-S. Characterization of the thermaltolerant mutants of *Chlorella sp*. with high growth rate and application in outdoor photobioreactor cultivation. **Bioresource Technology**, 101, 2880–2883, 2010.

PATIAS, L.D., FERNANDES, A. S., PETRY, F.C., MERCADANTE, A.Z., JACOB-LOPES, E., ZEPKA, L.Q. Carotenoid profile of three microalgae/cyanobacteria species with peroxyl radical scavenger capacity. **Food Research International**, 100, 260-266, 2017.

PELROY, R.A., BASSHAM J.A., Photosynthetic and dark carbon metabolism in unicellular blue-green algae. **Archives of Microbiology.** 86(1):25-38, 1972.

PENG, J., YUAN, J.-P., WU, C.-F., WANG, J.-H. Fucoxanthin, a Marine Carotenoid Present in Brown Seaweeds and Diatoms: Metabolism and Bioactivities Relevant to Human Health. **Marine Drugs**, 9, 1806–1828, 2011.

PEREZ-GARCIA, O., ESCALANTE, F. M. E., DE-BASHAN, L. E., and BASHAN, Y. Heterotrophic cultures of microalgae: metabolism and potential products. **Water Research**. 45, 11–36, 2011.

POOJARY, M.M., BARBA, F.J., FALIAKBARIAN, B., DONSÍ, B., PATARO, G., DIAS, D.A., JULIANO, P. Innovative alternative technologies to extract carotenoids from microalgae and seaweeds. **Marine Drugs**. 14 (11), 214, 2016.

PŘIBYL, P.; PILNÝ, J.; CEPÁK, V.; KAŠTÁNEK, P. The role of light and nitrogen in growth and carotenoid accumulation in *Scenedesmus* sp. **Algal Research**, 16, 69-75, 2016.

QUEIROZ, M. I. et al. Single-cell oil production by cyanobacterium Aphanothece microscopica Nägeli cultivated heterotrophically in fish processing wastewater. **Applied Energy**, v.88, n.10, p.3438–3443, 2011.

QUEIROZ, M. I.; JACOB-LOPES, E.; ZEPKA, L. Q.; BASTOS, R. G.; GOLDBECK, R. The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor. **Bioresource Technology**, v.98, p.2163–2169, 2007.

QUEIROZ, M.I. et al. Evaluación Del crecimiento de la *Aphanothece microscopica Nägeli* en las aguas residuales de la parbolizacion del arroz. **Revista información Tecnológica**, v.1, p.61–65, 2002.

RAMÍREZ-MÉRIDA, L.G., ZEPA, L.Q., JACOB-LOPES, E. Produção atual de microalgas em escala industrial JCM Pires (Ed.), Recentes Avanços em Energia Renovável , Betham Science Publishers. 242 – 260, 2017.

RIZWAN, M., MUITABA, G., MEMON, S.A., LEE, K, RASHID, N. Exploring the potential of microalgae for new biotechnology applications and beyond: A review. **Renewable Sustainable Energy Reviews**, 92, 394-404, 2018.

RODRIGUES, D. B.; FLORES, É. M. M.; BARIN, J. S.; MERCADANTE, A. Z.; JACOB-LOPES, E.; ZEPKA, L. Q. Production of carotenoids from microalgae cultivated using agroindustrial wastes. **Food Research International**, 65, 144-148, 2014.

RODRIGUES, D.B.; MENEZES, C. R.; MERCADANTE, A.; JACOB-LOPES, E.; ZEPKA, L.Q. Bioactive pigments from microalgae *Phormidium autumnale*. **Food Research International**, 77, 273-279, 2015.

RODRIGUEZ-AMAYA, D.B. Food Carotenoids: Chemistry, Biology, and Technology. 1st ed. **John Wiley & Sons, Ltd;** p. 306p. ISBN 978-1-118-73330-, 2016.

RODRIGUEZ-AMAYA. Status of carotenoid analytical methods and in vitro assays for the assessment of food quality and health effects. **Food Chemistry and Biochemistry**, v.1, p.56-63, 2015.

RODRIGUEZ-GARCIA, I., GUIL-GUERRERO, J.L. Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. **Food Chemistry**, 108,1023–26, 2008.

RUSSO, D. A. M. T. Estudo do crescimento da microalga *Chlorella vulgaris* numa água residual tratada, sob diferentes condições de fotoperíodo e temperatura. **Dissertação. (Mestrado em Energia e Bioenergia)** – **Universidade Nova de Lisboa, Lisboa.**111f**,** 2011.

SAFAFAR, H., ULDALL NØRREGAARD, P., LIUBIC, A., MØLLER, P., LØVSTAD HOLDT, S., JACOBSEN, C. Enhancement of protein and pigment content in two *Chlorella* species cultivated on industrial process water, **Journal of Marine Science and Engineering**, 4 (4), 84, 2016.

SAFI, C., ZEBIB, B., MERAH, O., PONTALIER, P.-Y., VACA-GARCIA, C. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. **Renewable and Sustainable Energy Reviews**, 35, 265–278, 2014.

SÁNCHEZ, A., CÉRON, M.C., GARCÍA, F., MOLINA, E., CHISTI, Y. Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture. **Enzyme and Microbial Technology**, 31,1015–23, 2002.

SANTOS, A. B. et al. Biogeneration of volatile organic compounds produced by *Phormidium autumnale* in heterotrophic bioreactor. **Journal of Applied Phycology**, v.28, n.2, p.1561–1570, 2016

SHI, X-M., ZHANG, X-W., CHEN, F. Heterotrophic production of biomass and lutein by *Chlorella* protothecoides on various nitrogen sources. **Enzyme and Microbial Technology**. 27,312–318, 2000.

SILVA, C.M.; FERREIRA, A.F.; DIAS, A.P.; COSTA, A.; A comparison between microalgae virtual biorefinery arrangements for bio-oil production based on lab-scale results. **Journal of Cleaner Production**, In Press, 2015.

SINGH, B.B.; SHAKIL, N. A.; KUMAR, J.; WALIA, S.; KAR, J. Develment of slow release formulations of β-carotene employing amphiphilic polymers and their release kinetics study in water and different pH conditions. **Journal of Food Science and Technology**, 52, 8068–8076, 2015.

SUGANYA, T., VARMAN, M., MASJUKI, H.H., RENGANATHAN, S. Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. **Renewable and Sustainable Energy Reviews**, 55, 909-941, 2016.

TAKAICHI, S. Carotenoids in algae: distributions, biosyntheses and functions. **Marine Drugs,** 9, 1101-1118, 2011.

TAKASHIMA, M., SHICHIRI, M., HAGIHARA, Y., YOSHIDA, Y., NIKI, E. Capacity of fucoxanthin for scavenging peroxyl radicals and inhibition of lipid peroxidation in model systems. **Free Radical Research**, 46, 1406–1412, 2012.

VALDERRAMA, J.O., PERRUT, M., MAJEWSKI, W. Extraction of astaxanthine and phycocyanine from microalgae with supercritical carbon dioxide. **Journal of Chemical & Engineering Data**,48, 827–30, 2003.

WANG, J., CHEN, S., XU, S., YU, X., MA, D., HU, X., CAO, X. (2012). *In Vivo* Induction of Apoptosis by Fucoxanthin, a Marine Carotenoid, Associated with Down-Regulating STAT3/EGFR Signaling in Sarcoma 180 (S180) Xenografts-Bearing Mice. **Marine Drugs**, 10, 2055–2068, 2012.

WANG, L., PAN, B., SHENG, J., XU, J., HU, Q. Antioxidant activity of *Spirulina platensis* extracts by supercritical carbon dioxide extraction. **Food Chemistry**, 105, 36–41, 2007.

WU, H., NIU, H., SHAO, A., CHENG., WU, C., DIXON, B.J., ZHANG, J.,YANG, S., WANG, Y. Astaxanthin as a Potential Neuroprotective Agent for Neurological Diseases. **Marine Drugs**, 13, 5750-5766, 2015.

WU, W., WANG, P-H., LEE, D-J., CHANG, J-S. Global optimization of microalgae-to-biodiesel chains with integrated cogasification combined cycle systems based on greenhouse gas emissions reductions. **Applied Energy**, 197, 63-82, 2017.

YAMADA, T., SAKAGUCHI, K. Comparative studies on *Chlorella* cell walls: induction of protoplast formation. **Archives of Microbiology**. 132, 10–13, 1982.

ZAGHDOUDI, K.; PONTVIANNE, S.; FRAMBOISIER, X.; ACHARD, M.; KUDAIBERGENOVA, R.; AYADI-TRABELSI, M.; GUIAVARC'H, Y. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and apricot (*Prunus armeniaca* L.). **Food chemistry**, 184, 131-139, 2015.

ZHAN, J.; RONG, J.; WANG, Q. Mixotrophic cultivation, a preferable microalgae cultivation mode for biomass/bioenergy production, and bioremediation, advances and prospect. **International Journal of Hydrogen Energy**, v. 42, n. 12, p. 8505-8517, 2016.

ZHANG, L., WANG, H., FAN, Y., GAO, Y., LI, X., HU, Z., DING, K., WANG, Y., WANG, X. Fucoxanthin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE and Nrf2-autophagy pathways. **Nature Scientific Reports**, *7*, 46763, 2017.

ZHANG, W., WANG, J., WANG, J., LIU, T. Attached cultivation of *Haematococcus pluvialis* for astaxanthin production. **Bioresource Technology**. 158, 329–335, 2014.